Drug Discovery Researches on Modulators of Lysine-Modifying Enzymes Based on Strategic Chemistry Approaches

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Enzymatic and post-translational modifications (PTMs) such as ubiquitination, acetylation, and methylation occur at lysine residues. The PTMs play critical roles in the regulation of the protein functions, and thus, various cellular processes. In addition, aberrations of the PTMs are associated with various diseases, such as cancer and neurodegenerative disorders. Therefore, we hypothesized that modulation of the PTMs and normalization of the PTM abnormalities could be useful as methods to control various cellular mechanisms and as a therapeutic strategy, respectively. To modulate the PTMs, we have focused on lysine-modifying enzymes and have pursued drug discovery researches on ubiquitination inducers, lysine deacetylase (KDAC) inhibitors, and lysine demethylase (KDM) inhibitors. For the identification of the modulators, we have used not only conventional drug design, such as structure-based drug design (SBDD) and ligand-based drug design (LBDD), but also “strategic chemistry approaches,” such as drug design based on enzyme catalytic mechanism. As a result, we have identified several modulators which have pharmacological effects in animal models or in cellular studies. In this review, focusing on the drug design based on enzyme catalytic mechanism, our drug discovery researches have been discussed.

Key words protein knockdown; deacetylase; demethylase; ubiquitin ligase; enzyme inhibitor; proteolysis-targeting chimera (PROTAC)

1. Introduction

Post-translational modifications (PTMs) are vital for cellular functions, e.g., maintenance of cell homeostasis, differentiation, and death. Proteins are synthesized by transcription and translation based on the DNA sequence. However, the proteins, as they are, do not exist or work; they gain stability and some functions, or trigger cellular responses by the PTMs. For example, phosphorylation at serine/threonine/tyrosine residues, which is one of the most common PTMs, transmits signals throughout the cell, and glycosylation at serine/threonine/asparagine residues in the endoplasmic reticulum (ER) is used for monitoring the status of the protein folding and for facilitating the trafficking of the proteins to the Golgi. Thus, PTMs control various cellular processes. In addition, they are deeply associated with the pathogenesis of various diseases. In cancer cells, abnormalities of the PTMs are observed, and the state of unfavorable PTMs in nerve cells causes neurodegenerative disorders. Thus, we envisioned that (i) we can control the cellular processes by chemical modulation of the related PTMs and (ii) artificial correction of the PTM aberrations could be used as therapeutic strategies. Indeed, we have been interested in ubiquitination, acetylation, and methylation at lysine residues, and we have attempted to identify small molecules which modulate the lysine modifications.

Lysine ubiquitination, acetylation, and methylation are enzymatically controlled (Fig. 1). In living cells, the ubiquitination is generally mediated by the sequential reactions of ubiquitin-activating enzyme (E1), conjugating enzyme (E2), and ligase (E3)22–25. On the contrary, lysine acetylation is reversibly regulated by lysine acetyltransferases (KATs) and deacetylases (KDACs).26–28 Lysine methylation is also governed by two kinds of enzymes, lysine methyltransferases (KMTs) and demethylases (KDMs); however, the enzymatic regulation is complicated because lysine residues can be mono-, di-, or trimethylated.20,29,30 Accordingly, the use of small molecular modulators against the lysine-modifying enzyme enables us to manipulate the PTMs and the resulting cellular processes (Fig. 1). From these perspectives, we have discovered small molecules as ubiquitination inducers, KDAC inhibitors, and KDM inhibitors, and performed their chemical biology studies or application studies aiming at drug development. In order to identify such small molecules, we have handled several drug discovery methodologies. If the threedimensional structures of target enzymes are available, we have used structure-based drug design (SBDD).31,32 On the
contrary, when we did not gain enough information on the
target enzyme structure, we have designed enzyme inhibitors
by ligand-based drug design (LBDD), focusing on structures
of the enzyme substrates or other lead compounds. In
addition to the conventional drug design, we have also ex-
ploited “strategic chemistry approaches,” which are drug
discovery methodologies based on chemical ideas, e.g., drug design based on enzyme catalytic mechanism, compound library construction by click chemistry, and application of N–C–H···O hydrogen bond to drug design (Fig. 2). The combinations of these methodologies have allowed us to effectively discover various small molecules as ubiquitination inducers, KDAC inhibitors, or KDM inhibitors. In particular, some of them have guided the development of first-in-class pharmacologic agents, which are currently under clinical trials. Here, we review our drug discovery studies on some of the modulators for the lysine-modifying enzymes, identified by the “strategic chemistry approaches,” especially drug design based on enzyme catalytic mechanism (Figs. 2A, B). This review discusses details of the design and biological evaluation of the identified small molecules in the following sections: ubiquitination (section 2), acetylation (section 3), and methylation modulators (section 4).

2. Ubiquitination Modulators and Their Application

Ubiquitin is a small protein consisting of 76 amino acids with a molecular weight of about 8.6 kDa. The ubiquitination, where the small protein is attached to proteins, is important for various biological processes, especially protein degradation. In general, poly-ubiquitinated proteins are degraded by proteasomes. In other words, the ubiquitin molecule works as a tag for proteins to be degraded. On the contrary, it has been reported that accumulation of ubiquitinated proteins is detected in Alzheimer’s, Parkinson’s, and Huntington’s diseases. Inhibition of the protein ubiquitination might be effective in the treatment of these diseases. Therefore, small molecules which modulate protein ubiquitination should be useful for the control of protein degradation and for the treatment of ubiquitin-related diseases. So far, we have reported small molecules which induce ubiquitination of targeted proteins and E1 inhibitors, especially, the former, which are often called proteolysis-targeting chimeras (PROTACs) or specific and nongenetic inhibitor of apoptosis proteins (IAP)-dependent protein erasers (SNIPERs), have recently attracted attention as tools for chemical protein knockdown. We have contributed to the development of these molecules in their early stages. In this section, their design and application studies are presented.

We strategically designed small molecules based on the physiological protein ubiquitination mechanism, called the ubiquitin–proteasome system (UPS) (Fig. 3A). In UPS, the protein ubiquitination is mediated by three kinds of enzymes E1, E2, and E3. First, E1 activates the ubiquitin by using ATP to form a ubiquitin–E1 conjugate via a thioester linkage between the C-terminal carboxylate of ubiquitin and the catalytic cysteine of E1. Then, the ubiquitin is transferred from E1 to E2 through a thioester exchange reaction to obtain a ubiquitin–E2 conjugate. Finally, E3 recognizes the substrate protein and completes protein ubiquitination by transferring the ubiquitin from E2 to the substrate protein. The reactions occur repeatedly to generate polyubiquitinated proteins that are recognized and degraded by proteasomes. In UPS, E3 determines the proteins to be ubiquitinated. Therefore, we speculated that if E3 and the protein of interest (POI) could be close to each other or could form an artificial (non-physiological) complex of E3 with the POI, the POI should be ubiquitinated by E3, as E3 physiologically ubiquitinates proteins that are bound to it (Fig. 3B). Based on this hypothesis, we designed hybrid small molecules by conjugating an E3 ligand to a ligand of the POI. Such hybrid small molecules were expected to induce the ternary complex and the POI ubiquitination by the recruitment of ubiquitin molecules from E2 (Fig. 3B). The generated POI with a poly-ubiquitin chain should be ultimately degraded by proteasomes as well as physiologically ubiquitinated proteins (Fig. 3B). Accordingly, the hybrid small molecules could work as ubiquitination inducers and protein degraders, and we could knockdown the POI chemically and post-translationally by using them.

In the proof of concept study, we used bestatin analogs as E3 ligands and all-trans retinoic acid (ATRA) as a POI ligand (Fig. 4). The bestatin analogs can bind cIAP1, which is a member of the IAP with E3 ligase activity. On the contrary, ATRA can bind to cellular retinoic acid-binding proteins (CRABPs), although it is a well-known endogenous ligand of retinoic acid receptors (RARs), which are well-known endogenous ligands of retinoic acid receptors (RARs). The introduction of a substituent at the C4 position of ATRA does not affect the binding affinity for CRABPs, but the modification causes a decrease in ATRA’s binding affinity for RARs. Thus, we designed and synthesized bestatin-ATRA hybrid compounds as small molecules which induce ubiquitination/degradation of CRABP (CRABP SNIPERs) (Fig. 4). The treatment of cells expressing cIAP1 with CRABP SNIPERs selectively decreased the levels of CRABPs without affecting RAR levels. Mechanistic analysis of the decrease in CRABP by the SNIPERs suggested that the SNIPERs induces (i) the formation of a complex between cIAP1 and CRABP, (ii) cIAP1-dependent CRABP ubiquitination in the presence of the SNIPERs, and (iii) proteasomal degradation of CRABP. Furthermore, we applied the SNIPERs to chemical biology
study and uncovered that chemical degradation of CRABP-II inhibits the migration of neuroblastoma cells and has synergistic effects on the inhibition of cancer cell growth with caspase activation. In addition, replacement of the bestatin analogs with other IAP ligands was possible and the replaced compounds worked as POI ubiquitination/degradation inducers. 

Since this methodology was successful, it has been widely used and applied to target several proteins. In addition, not only IAP ligands but also other E3 ligands have been used for protein knockdown, where some of them are especially adaptable to in vivo studies or to the clinical trial stages. Thus, our drug discovery studies on ubiquitination inducers have driven the establishment of protein degradation approach using small molecules. At the end of this section, I briefly emphasize some features of the ubiquitination/degradation inducers. First, they can decrease the protein levels, whereas, conventional drugs such as enzyme inhibitors and receptor antagonists inhibit only the protein functions. This may be an advantage in case of targets which not only have enzymatic/receptor-like functions but also work cooperatively by interacting with other proteins (Fig. 5). Second, the ubiquitination/degradation inducers can work catalytically and indirectly induce inhibition by controlling protein levels (Fig. 5). Conventional drugs have only a stoichiometric effect, whereas, a molecule of the ubiquitination/degradation inducer can degrade more than one molecule of targeted proteins. This catalytic manner may provide high efficacy, even at low concentrations, which decreases the off-target and side effects. Third, chemical protein knockdown can be applied to various proteins, including undruggable proteins. In fact, CRABP, which is not an enzyme or receptor, was an undruggable protein; although CRABPs could be target proteins for the treatment of some diseases, compounds which directly control the function(s) of CRABPs had never been reported. Thus, this approach may expand the range of therapeutic targets. Fourth, POIs are non-genetically degraded by small molecules. Genetic methods such as RNA interference (RNAi) and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system where nucleic acid-based reagents are required have several limitations, including poor stability in serum, poor tissue/cell penetration, and low bioavailability. In general, small molecules have the required stability and cell membrane permeability to be applied to in-cell or in vivo studies. Therefore, protein knockdown could be a simple and easy technique to complement genetic ablation at the DNA level or the mRNA level. The details of these features have been described in the previous reviews. 

3. Lysine Acetylation Modulators and Their Application

Protein acetylation occurs at the lysine residues of various proteins. In the physiological condition, the ε-amino group of the lysine residues is protonated and obtains a cationic charge. On the contrary, an acetyl amide group does not have any charge. Therefore, protein acetylation removes the cationic charge from the lysine residues. In addition, there is a difference between the amino group (including the cationic state) and the acetyl amide group in the patterns of hydrogen-bond formation with amino acid residues. Since acetylation induces changes in the charge state or in the hydrogen bond pattern at the lysine residues, it can govern the protein structure, interaction with other proteins, and molecular recognition. Among the protein acetylations, histone acetylation is well-studied and is known to be involved in transcriptional activation, chromatin remodeling, and DNA repair. Tubulin and cortactin, which are cytoplasmic proteins, are also the representative proteins to be acetylated. Tubulin acetylation is thought to be related to microtubule stabilization. Cortactin acetylation is reported to control its location; acetylated cortactin is mostly localized in the nucleus, but the deacety-
lation increases the binding of cortactin to Keap1 in the cytoplasm to inhibit cell migration.83–85) The protein acetylation levels are controlled by a balance of KATs and KDACs.26–28) Therefore, we have been interested in the accumulation of acetylated proteins by KDAC inhibition and pursued drug discovery studies on KDAC inhibitors.20,21,38,86–88)

Till date, 18 KDACs have been identified.89–92) They can be divided into two categories: 11 zinc-dependent deacetylases (called histone deacetylases: HDAC1–11) and 7 oxidized form of nicotinamide adenine dinucleotide (NAD\(^+\))-dependent deacetylases (called sirtuins: SIRT1–7). Since each KDAC isozyme is thought to have specific substrate proteins/lysine residues and to be associated with distinct diseases, isozyme-selective KDAC inhibitors are of interest as chemical tools and therapeutic agents with few side effects.93) In our group, we have reported several isoform-selective inhibitors against HDAC3,40,94) HDAC6, 95,96) HDAC8, 36,41) and SIRT2 39,97–99) by SBDD, LBDD, strategic chemistry approaches, and their combination. In this section, I present the studies on SIRT2-selective inhibitors identified by combining SBDD with drug design based on an enzymatic mechanism.98,99)

SIRT2 is classified as a cytoplasmic NAD\(^+\)-dependent deacetylase100) and is involved in the destabilization of microtubules through deacetylation of acetylated \(\alpha\)-tubulin.101) Although the functions of SIRT2 are not completely understood yet, they have been suggested to be associated with some diseases, including neurodegenerative disorders.91,102–105) Therefore, SIRT2-selective inhibitors are of interest as tools for elucidating the biological functions of the enzyme and as potential therapeutic agents for neurodegenerative diseases. Several classes of small molecular SIRT2-selective inhibitors have been identified so far.106) However, their use is limited because their cellular activity is not strong enough to be tested in cells. In other words, stronger SIRT2 inhibitors were needed.

In 2012, we reported NCO-90 as a SIRT2 selective inhibitor with an IC\(_{50}\) value within micromolar range97) and recently, we have succeeded in determining a crystal structure of SIRT2 in complex with NCO-9098) (Figs. 6A, B). As we scrutinized the structure, we found that NCO-90 binds to the “selectivity pocket”107) (Figs. 6A, B). The pocket is located near the NAD\(^+\) and acetyl lysine substrate binding pocket. Based on these findings, we started the structural optimization of NCO-90 by SBDD. After several compounds were tested, we finally designed KPM-1 by conjugating NCO-90 with the lysine moiety. We expected KPM-1 to exhibit a more potent and selective SIRT2-inhibitory activity because it occupied the “selectivity pocket” and substrate binding pockets (Figs. 6C–E). We synthesized KPM-1 and evaluated its SIRT2-inhibitory activity and SIRT2-selectivity in fluorescence assays,108) and found that KPM-1 strongly inhibited SIRT2 without inhibiting other SIRTs (Fig. 6C). This result supported the hypothesis that targeting both the substrate binding pocket and the “selectivity pocket” represents a useful strategy to develop novel SIRT2 inhibitors.98) Inspired by this result, we also designed mechanism-based SIRT2 inhibitors.

The catalytic mechanism of deacetylation by SIRT2 has been proposed as follows (Fig. 7A); first, the amide oxygen of the acetylated lysine substrate nucleophilically attacks the \(1^st\)-carbon of the ribose ring of NAD\(^+\) and nicotinamide is released; next, the resulting imido ester is attacked by the \(2^nd\)-OH group of the ADP ribose activated by the histidine residues; finally, hydrolysis of acetal affords \(2^nd\)-O-acetyl-ADP-ribose and the deacetylated lysine109,110) (Fig. 7A). This proposed mechanism drove us to design KPM-2 by replacing the amide oxygen of KPM-1 with a sulfur atom98,105,111) (Figs. 7B, C). Like the acetylated lysine substrate, the thioamide of KPM-2 can attack NAD\(^+\) at the active site of SIRT2105,111) (Figs. 7B, C). We synthesized KPM-2 and evaluated its SIRT2-inhibitory activity and SIRT2-selectivity in fluorescence assays,108) and found that KPM-2 strongly inhibited SIRT2 without inhibiting other SIRTs (Fig. 6C). This result supported the hypothesis that targeting both the substrate binding pocket and the “selectivity pocket” represents a useful strategy to develop novel SIRT2 inhibitors.98) Inspired by this result, we also designed mechanism-based SIRT2 inhibitors.

Fig. 6. (A) X-Ray Structure of SIRT2 in the Complex with NCO-90 (PDB Code: 5Y5N); (B) Schematic Diagram of Fig. 6A; (C) Drug Design of SIRT2-Selective Inhibitor KPM-1 by Conjugation of NCO-90 with the Acetyl Lysine Substrate and SIRT-Inhibitory Activities of NCO-90 and KPM-1; (D) Schematic Diagram of the Binding Mode of the Acetyl Lysine Substrate; (E) Schematic Diagram of Plausible Binding Mode of KPM-1 to SIRT2
exhibit stronger SIRT2-inhibitory activity. Indeed, the SIRT2-inhibitory activity of KPM-2 was greater than that of KPM-1 with a high selectivity (Fig. 7C). Mechanistic studies by enzyme kinetics and MALDI-MS analysis suggested that the SIRT2-inhibition by KPM-2 was mediated by the formation of the KPM-2/ADP-ribose conjugate, suggesting that KPM-2 is a mechanism-based inhibitor targeting the three pockets\(^{98}\) (Fig. 7B).

Furthermore, we applied KPM-2 to the cellular system and evaluated its effect on protein acetylation in the cell. Treatment of breast cancer cell line MDA-MB-231 cells expressing SIRT2 with KPM-2 induced the accumulation of acetylated α-tubulin, which is one of the SIRT2 substrates. This result suggests that KPM-2 increases the α-tubulin acetylation levels via SIRT2 inhibition. Moreover, SIRT2 has received increasing attention due to its potential involvement in neurodegenerative diseases.\(^{102–104}\) Therefore, we examined the effect of KPM-2 on the neurite outgrowth of Neuro-2a (N2a) cells and demonstrated that KPM-2 promoted neurite outgrowth of N2a cells, but its activity was limited. Then, we performed structural optimization of KPM-2 and designed compound 1 by conversion of the peptidyl moiety to a diketopiperazine scaffold, which was expected to form hydrogen bonds with amino acid residues in the catalytic site, like the peptide bonds of the substrate\(^{99}\) (Figs. 6D, 7C). Although SIRT2-inhibitory activity of 1 was weaker than that of KPM-2 (Fig. 7C), it was a mechanism-based inhibitor. Interestingly, the neurite outgrowth activity of 1 was stronger than that of KPM-2, which could be due to the pseudopeptidic structure and relatively low membrane permeability of KPM-2. These findings suggest that SIRT2 inhibition and the following accumulation of acetylated protein might be a novel therapeutic strategy for neurodegenerative disorders.

### 4. Lysine Methylation Modulators and Their Application

A methyl group, whose molecular weight is only 15 Da, is the smallest substitute in the alkyl groups or even in functional groups added to proteins as PTMs. However, this group, upon addition, has a large potential of altering the chemical property or molecular recognition of proteins.\(^{112–115}\) In addition, there are three types of methylation states for each of the lysine residues, mono-, di-, and tri-methylation. Multiple lysine methylations are complicated, but the control system for protein methylation is interesting. For example, monomethylated lysine 9 of histone H3 (H3K9me1) and H3K27me1 are abundant at active gene promoters, whereas H3K9me3 and H3K27me3 are associated with transcriptionally inactivated gene promoters.\(^{116–119}\) Another example is K382me1/me2 on p53, where monomethylation promotes transcriptional repression of the p53 target genes, whereas dimethylation activates the p53-dependent gene expression.\(^{120,121}\) The complicated lysine methylations are reversibly governed by KMTs and KDMs.\(^{29,30,122}\) Therefore, to modulate the protein methylation, we have focused on KDMs and identified their inhibitors.\(^{20}\)

The KDMs are divided into two classes on the basis of their structures and catalytic mechanisms.\(^{19,30,122}\) One class is composed of FAD-dependent oxidases, namely, lysine-specific demethylase 1 (LSD1: also called KDM1A) and LSD2 (KDM1B).\(^{123,124}\) The other class is a family of Fe(II)/α-ketoglutarate (αKG)-dependent oxidases (KDM2–8).\(^{125}\) So far, we have identified LSD1\(^{14}\), LSD2,\(^{32,35,126–128}\) KDM2/7,\(^{129}\) and KDM5\(^{11,130–132}\) inhibitors by LBDD, SBDD, and strategic chemistry approaches. In this section, we discuss the discovery of LSD1 inhibitors based on SBDD, LBBD, and enzyme catalytic mechanism.\(^{126}\)

The LSD1 regulates gene expression and protein stability by
catalyzing the demethylation of histone and non-histone proteins. It is also thought to be a target for several diseases, such as neuroblastoma and leukemia. Therefore, LSD1 inhibitors are of interest as chemical tools for studying the functions of LSD1 and as candidate therapeutic agents. However, potent and selective LSD1 inhibitors had not been reported when we started studies on LSD1 inhibitors. Earlier, trans-2-phenylcyclopropylamine (PCPA/Tranylcypromine), which was originally found as an inhibitor of monoamine oxidases (MAOs; also FAD-dependent enzymes), was the best-studied

Fig. 8. (A) Proposed Catalytic Mechanism for the Demethylation of Methylated Lysines by LSD1; (B) Proposed Mechanism of LSD1 Inhibition by PCPA

Fig. 9. (A) Model of LSD1, MAO A, and MAO B Inhibition by PCPA; (B) Model of the Expected Mechanism of LSD1-Targeted Delivery of PCPA by 2 and Its LSD1-Selective Inhibition
LSD1 inhibitor and was frequently used for biological studies. However, PCPA has poor intracellular activity, insufficient inhibitory potency, or inadequate selectivity for LSD1 over MAOs. To overcome these issues, we conducted drug discovery researches based on the idea that LSD1 could be potently and selectively inhibited by delivering PCPA directly to the LSD1 active site.

To identify the series of LSD1 inhibitors, we focused on LSD1 catalytic mechanism and inhibitory mechanism of PCPA. LSD1 catalyzes oxidative demethylation of mono- or di-methylated lysine, FAD-dependently, to obtain the iminium cation. Then, the iminium cation is hydrolyzed to produce the demethylated lysine. First, FAD extracts one electron from the nitrogen atom of PCPA in the catalytic site to form a cation radical. Then, the cyclopropyl ring of the radical species opens, followed by covalent bond formation with FAD. The FAD/PCPA conjugate stays in the catalytic site and inhibits LSD1 irreversibly. In the course of LSD1 inhibition, the nitrogen atom of PCPA is released as an ammonia molecule through hydrolysis of the imine intermediate. Considering this mechanism, together with our idea of delivering PCPA directly to the LSD1 active site, we designed LSD1 inhibitors, in which PCPA is coupled to a lysine carrier moiety (Figs. 9, 10).

Based on this hypothesis, we synthesized and evaluated several conjugates of the PCPA moiety with lysine derivatives by SBDD and LBDD approaches and identified NCD-25 and NCD-38 as highly potent and selective LSD1 inhibitors (Fig. 11). NCD-25 and NCD-38 inhibited LSD1 with IC50 values of 0.48 and 0.59 \( \mu \text{M} \), respectively, whereas they did not inhibit MAOs at 100 \( \mu \text{M} \). Mechanistic analysis suggests that they inhibited LSD1 by drug delivery mechanism (Figs. 9B, 10B). In addition, NCD-38 increased the levels of H3K4me2, which is one of the LSD1 substrates in the cells, and induced the differentiation of leukemia cells. Furthermore, we found that NCD-38 caused the eradication of leukemia cells in vivo. Thus, NCD-38 was considered as a therapeutic agent. Although NCD-38 has never reached clinical stages, further structural optimization of NCD-38 led to IMG-7289 (Fig. 11), which is being clinically tested for the treatment of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) in the United States and Australia (phase I/II). Moreover, we identified some mechanism-based LSD1 inhibi-

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Fig. 10. (A) Mechanism of LSD1 Inhibition by PCPA; (B) Putative Mechanism of LSD1-Targeted Delivery of PCPA by 2
tors, including peptide-based LSD1 inhibitors\textsuperscript{145–148} and the drug delivery molecules were applied to PCPA–drug conjugates,\textsuperscript{149–151} which release drugs by LSD1 inhibition. Thus, we identified several LSD1 inhibitors by strategic design focusing on LSD1 catalytic mechanism.

5. Conclusion
As discussed in this review, we have been interested in the artificial modulation of PTMs, especially lysine modifications for regulating various biological processes or for therapeutic strategy and we have challenged the exploration of modulators for lysine-modifying enzymes. In our drug discovery studies, as well as conventional drug design methods, we have utilized “strategic chemistry approaches,” such as drug design based on enzyme catalytic mechanism. The approaches have led us to identify several modulators, such as ubiquitination/degradation inducers, KDAC inhibitors, and KDM inhibitors. In addition, we have performed biochemical experiments aiming at drug development. Among them, the researches on the ubiquitination/degradation inducers and the KDM inhibitors have promoted new drug developments.\textsuperscript{44–46} The drugs which are currently at clinical trial stages may be approved and may be delivered to many patients in the future.

Our drug discovery studies based on “strategic chemistry approaches” have been successful in the pharmaceutical science field. The achievements have further accelerated our research and we are currently performing drug discovery studies on modulators for lysine-modifying enzymes based on enzyme inhibition kinetics\textsuperscript{152–155} and in situ click chemistry.\textsuperscript{156–159} The findings will be presented as articles in the near future.

The lysine modifications are not limited to ubiquitination, acetylation, and methylation. For example, lysine modifications by other acyl groups\textsuperscript{150–162} have also received great attention; few selective modulators for these modifications are known. In addition, the protein modifications are only a part of chemical modification of biomolecules in physiological condition. As well as DNA methylation,\textsuperscript{163–165} which has long been studied, active researches on RNA modifications, some of which are enzymatically regulated, have also been carried out recently.\textsuperscript{166–170} I intend to expand our research to the modulation of other PTMs or RNA modifications with the help of novel “strategic chemistry approaches.” I hope that the researches described in this review or our current/future works provide small molecules, which will be used not only as tools for the detailed elucidation of the biological functions of the modification but also as novel therapeutic agents for several diseases.

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