

Lysine-Specific Histone Demethylases 1/2 (LSD1/2) and Their Inhibitors



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Contents

1	Introduction	198
2	Biology of Lysine-Specific Histone Demethylases 1/2 (LSD1/2)	199
3	Structural Studies and Catalytic Mechanism of LSD1/2	200
4	Link of LSD1/2 to Diseases	200
4.1	Link of LSD1/2 to Cancer	201
4.2	Link of LSD1 to Viral Infection	201
4.3	Link of LSD1 to Globin Disorders	202
4.4	Link of LSD1 to Metabolic Diseases	202
4.5	Link of LSD1 to Central Nervous System (CNS) Disorders	203
5	LSD1 Inhibitors and Their Biological/Therapeutic Applications	203
6	Summary	214
	References	214

Abstract Histone lysine methylation, one of the epigenetic mechanisms, plays a pivotal role in various biological events, including cell cycle progression, immune response, and signal transduction. Histone methylation is closely associated with the oncogenesis and proliferation of cancer cells, and its alteration has been identified in many cancer cells. In addition, histone methylation is involved in such non-cancerous diseases as globin disorders and neurological disorders. Several enzymes that control histone methylation have been identified, including lysine-specific histone demethylases 1/2 (LSD1/2). As LSD1/2 are involved in various diseases, their inhibitors are considered useful not only as a chemical tool for probing the biology of LSD1/2 but also as therapeutic agents. In this chapter, the biology, pharmacology, and inhibitors of LSD1/2 are presented, and the potential of LSD inhibitors as therapeutic agents is discussed.

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Abbreviations

ADC	Antibody-drug conjugates
AML	Acute myelogenous leukemia
AR	Androgen receptor
ATRA	All- <i>trans</i> -retinoic acid
CNS	Central nervous system
DDS	Drug delivery systems
ER α	Estrogen receptor α
FAD	Flavin adenine dinucleotide
GSC	Glioma stem cells
HCF-1	Host cell factor-1
HSV	Herpes simplex virus
KDM	Lysine demethylase
KMT	Lysine methyltransferase
LSD	Lysine-specific histone demethylase
MAO	Monoamine oxidase
MDS	Myelodysplastic syndrome
NSCLC	Non-small cell lung cancer
PCPA	Phenylcyclopropylamine
PDC	PCPA-drug conjugate
SCLC	Small cell lung carcinoma
siRNA	Small interfering RNA
VZV	Varicella zoster virus

1 Introduction

Histone lysine methylation is one of the epigenetic mechanisms that regulate the expression of genes independently of the changes in DNA sequence. The methylation of histone (H) lysine (K) residues occurs at H1K26, H3K4, H3K9, H3K27, H3K36, H3K79, and H4K20 and is responsible for transcriptional activation as well as silencing [1, 2]. In addition, the ϵ -amino group of the lysine residues can undergo mono-, di-, or trimethylation, and this differential methylation gives functional diversity to each lysine methylation site. For example, the dimethylation of H3K4 occurs in both inactive and active genes, whereas the trimethylation is exclusive to active genes [3]. Similarly, the monomethylation of H3K9 is seen in active genes, whereas the trimethylation of H3K9 is associated with gene repression [4].

Histone lysine methylation is reversibly controlled by two kinds of enzymes, lysine methyltransferases (KMTs) and lysine demethylases (KDMs) [5]. KMTs add a methyl group to histone lysine residues, whereas KDMs remove the methyl group from methylated histone lysine residues, discriminating the methylated positions and states.

Lysine-specific histone demethylases 1/2 (LSD1/2) (KDM1A/B) are KDMs and are associated with several diseases, such as cancer and neurological disorders. Therefore, small-molecule inhibitors of LSD are of interest as potential therapeutic agents. In this chapter, the biology and pharmacology of LSD and hitherto reported LSD inhibitors are presented, and their potential as therapeutic agents is discussed.

2 Biology of Lysine-Specific Histone Demethylases 1/2 (LSD1/2)

Histone methylation had been regarded as an irreversible modification because of the high thermodynamic stability of the N–C bond. Indeed, whereas a number of KMTs had been identified by 2003 [1], no KDMs had been identified. However, in 2004, LSD1 was the first histone demethylase to be identified [6].

LSD1 removes the methyl groups from mono- and dimethylated Lys4 of histone H3 (H3K4me1/2) through flavin adenine dinucleotide (FAD)-dependent enzymatic oxidation (Fig. 1) [6]. In prostate cell lines, LSD1 also demethylates H3K9me1/2 and regulates androgen receptor (AR)-mediated transcription [7]. The targets of LSD1 regulatory demethylation are not limited to histone H3; LSD1 also demethylates nonhistone proteins, such as p53 [8], DNA methyltransferase 1 [9], STAT3 [10], E2F1 [11], and MYPT1 [12], and regulates their cellular functions.

LSD2 (KDM1B), the other flavin-dependent lysine demethylase, was found in 2009 and exhibits the same H3K4 demethylase activity as LSD1 [13]. However, the function and role of LSD2 are likely to be different from those of LSD1, although they remain unclear so far. It has been reported that LSD2 establishes maternal genomic

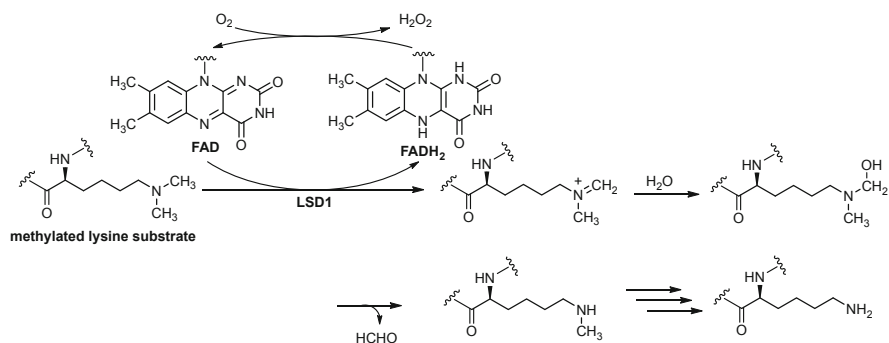


Fig. 1 Catalytic mechanism for the demethylation of methylated lysine substrate by LSD1

imprints, activates gene expression via H3K4 methylation [14], and possesses a demethylase-activity-independent repression function [15]. Recently, it has been reported that LSD2 possesses unexpected E3 ubiquitin ligase activity and inhibits lung cancer cell growth by promoting the ubiquitylation and degradation of *O*-linked *N*-acetylglucosamine transferase [16].

3 Structural Studies and Catalytic Mechanism of LSD1/2

The X-ray crystal structure of LSD1 complexed with CoREST and a histone H3 peptide was determined by Yang et al. [17]. This crystal structure was able to shed light on how histone H3 is recognized. The structural data revealed that histone H3 adopts three consecutive γ -turns, establishing a side chain spacing that places its N terminus in an anionic pocket comprised of Asn, Trp, and two Asp residues. The structural data also confirmed the positioning of the lysine methyl groups in sufficient proximity to FAD for FAD-mediated catalysis.

The crystal structures of LSD1 and the detailed analysis of the catalytic mechanism have led to a solid understanding of the catalytic mechanism for the demethylation of methylated lysine substrates by LSD1 (Fig. 1) [6, 17–19]. First, the methylated lysine substrate is converted into an iminium cation, presumably through a two single-electron oxidation reactions of the amine by FAD. Next, the addition of a water molecule to the iminium cation and the subsequent deformylation afford demethylated lysine. The FADH₂ generated in the first step is oxidized by molecular oxygen to FAD, which is utilized again for lysine demethylation. As would be expected from the mechanism, the demethylation by LSD1 is limited to mono- or dimethylated lysine; LSD1 cannot demethylate trimethylated lysine. This proposed catalytic mechanism for the demethylation of methylated lysine substrates provides a basis for the design of selective LSD1 inhibitors.

For the structural study of LSD2, Fang et al. characterized NPAC protein (also known as GLYR1) as an LSD2-specific cofactor that facilitates LSD2-mediated H3K4me1 and H3K4me2 demethylation [20]. They also determined the crystal structures of LSD2 alone and LSD2 in complex with the NPAC protein in the absence and presence of a histone H3 peptide. The structures revealed that the NPAC protein stabilizes the interaction between LSD2 and the histone H3 peptide, thus enhancing the enzymatic activity of LSD2 [21].

4 Link of LSD1/2 to Diseases

Whereas LSD1 is involved in many normal biological events, such as organogenesis [22, 23] and adipocyte differentiation [24], it is associated with several disease states as well, including cancer, viral infection, globin disorders, metabolic syndromes, and neurological disorders. In this section, the links of LSD1/2 to diseases are presented.

4.1 Link of LSD1/2 to Cancer

LSD1 is overexpressed in various cancer cells and tissues, such as neuroblastoma [25], prostate cancer [7, 26], breast cancer [27–29], lung cancer [30], and bladder cancer cells [30]. Furthermore, the results of RNAi-mediated knockdown or LSD1 inhibition suggest that this enzyme is associated with cancer cell growth by modulating pro-survival gene expression and p53 transcriptional activity [25, 27, 31].

Schulte et al. reported that LSD1 expression is correlated with adverse outcome in neuroblastic tumors [25]. The RNAi-mediated knockdown of LSD1 suppresses cell growth, and LSD1 inhibition results in growth inhibition of neuroblastoma cells in both in vitro and in vivo assays. In prostate cancer cells, AR binds an enhancer in the AR second intron and represses AR gene expression through LSD1 recruitment and H3K4me1/2 demethylation [32]. It was also reported that LSD1 is involved in breast cancer proliferation in estrogen receptor α (ER α)-dependent and ER α -independent manners [33]. Whereas LSD1 interacts with ER α in ER α -positive breast cancer cells [34], it is highly expressed also in ER-negative breast cancer cells, and the pharmacological LSD1 inhibition or knockdown of LSD1 using small interfering RNA (siRNA) results in the growth inhibition of ER-negative breast cancer cells and induces the regulation of several proliferation-associated genes, such as p21, ERBB2, and CCNA2 [28]. LSD1 expression is higher also in lung cancer tissue than normal lung tissue [35]. The overexpression of LSD1 protein is associated with shorter overall survival in non-small cell lung cancer (NSCLC) patients, and the interruption of LSD1 using siRNA or chemical LSD1 inhibition suppresses the proliferation, migration, and invasion of NSCLC A549, H460, and 293T cells. It has been reported that LSD1 is overexpressed in leukemia cells and is involved in leukemia cell proliferation and differentiation [36]. Importantly, LSD1 is closely associated with the growth of cancer cells with pluripotent stem cell properties expressing Oct4 and SOX2 [37–39].

It has also been reported recently that LSD2 is involved in breast cancer progression [40]. LSD2 protein level is significantly elevated in malignant breast cancer cell lines compared with normal breast epithelial cell lines. Whereas the overexpression of LSD2 in MDA-MB-231 cells significantly promotes cell proliferation, the siRNA-mediated knockdown of endogenous LSD2 inhibits the growth of multiple breast cancer cell lines, suggesting the critical role of LSD2 in the regulation of breast cancer progression.

4.2 Link of LSD1 to Viral Infection

LSD1 regulates viral gene transcription [41]. In herpes simplex virus (HSV) and varicella zoster virus (VZV), an increase in H3K4 methylation and a decrease in

H3K9 methylation are needed for viral gene transcription in a host cell [42]. To increase methylation, the virus recruits host cell factor-1 (HCF-1) and an HKMT complex. Kristie and co-workers showed that LSD1 interacts with the HCF-1 component of the HKMT complex and demethylates H3K9 [41]. They also showed that blocking LSD1 activity leads to the inhibition of viral gene transcription, suggesting that LSD1 inhibitors could work as anti-HSV and anti-VZV agents. In addition to HSV and VZV, LSD1 has also been reported to be involved in latent HIV infection [43] and hepatitis B virus-induced liver carcinogenesis [44].

4.3 Link of LSD1 to Globin Disorders

The human β -globin locus consists of embryonic, fetal, and adult globin genes that are expressed during development. Mutations in the globin locus result in β -globin disorders, such as β -globinopathies, β -thalassemia, and sickle cell disease. Although the fetal globin genes are autonomously silenced in adult-stage erythroid cells, mutations lying both within and outside the locus lead to natural variations in the level of fetal globin gene expression, and some of the mutations ameliorate the clinical symptoms of β -globin disorders. LSD1 is associated with fetal globin gene repression in adult-stage erythroid cells. LSD1 has been shown to interact with the transcription factor BCL11A through a complex containing CoREST [45] and to mediate part of BCL11A's strong γ -globin gene silencing activity. LSD1 also has been shown to interact with the TR2-TR4-DNMT1-LSD1 complex, along with several other corepressor complexes [46]. LSD1 inhibition results in increased γ -globin gene expression in β -globin locus-bearing transgenic mice and cultured primary human erythroid cells [45, 47], suggesting the effectiveness of LSD1 inhibitors as therapeutic agents for β -globin disorders.

4.4 Link of LSD1 to Metabolic Diseases

It has been suggested that LSD1 is involved in metabolic diseases [48]. LSD1 regulates energy-expenditure genes in adipocytes, and the loss of LSD1 function in adipocytes induces a number of regulators of energy expenditure and mitochondrial metabolism, resulting in the activation of mitochondrial respiration. The expression of LSD1-target genes is downregulated in the adipose tissues of mice on a high-fat diet as compared with that in tissues of mice on a normal diet. This downregulation is reverted by suppressing the function of LSD1, indicating the involvement of LSD1 in metabolic diseases.

4.5 *Link of LSD1 to Central Nervous System (CNS) Disorders*

LSD1 has been reported to be involved in central nervous system (CNS) disorders, such as depression and Alzheimer's disease. LSD1 regulates the expression of genes associated with cognitive function, neuroplasticity, and memory in senescence-accelerated SAMP8 mice [49]. LSD1 also controls the expression of genes related to immune reaction and inflammation, including S100A9, which is emerging as an important contributor to inflammation-related neurodegeneration.

It was reported that neuroLSD1, a dominant-negative splicing isoform of LSD1, is responsible for emotional behavior [50]. The knockout of neuroLSD1 in mutant mice reduces the expression of psychosocial-stress-induced genes, resulting in low anxiety-like behavior.

5 **LSD1 Inhibitors and Their Biological/Therapeutic Applications**

As mentioned above, LSD1 represents an interesting target for epigenetic drugs as supported by data related to its link to several diseases, including cancer, viral infection, globin disorders, metabolic diseases, and CNS disorders. Thus, expectations are high regarding the use of LSD1 inhibitors as therapeutic agents for cancer and non-cancer diseases. In this section, some of the previously reported LSD1 inhibitors (Fig. 2) and their potential as therapeutic agents are presented.

LSD1 is an amine oxidase that catalyzes the demethylation of mono- or dimethylated histone lysine residues and shows homology with monoamine oxidases (MAOs) A and B [51]. Indeed, *trans*-2-phenylcyclopropylamine (PCPA) (Fig. 2), a MAO inhibitor used as an antidepressant, was found to be also able to inhibit LSD1 and LSD2 [13, 51]. It was shown that PCPA is a mechanism-based irreversible inhibitor of LSD1. Kinetics, MS, and X-ray analysis data suggested that PCPA inhibits LSD1 through the formation of a covalent adduct with the flavin ring following one-electron oxidation and cyclopropyl ring opening (Fig. 3) [51, 52]. PCPA at high concentrations induces an increase of global H3K4 methylation and growth inhibition of neuroblastoma cells and bladder cancer cells [25, 53]. In addition, the combination of PCPA and all-*trans*-retinoic acid (ATRA) is an effective therapy for acute myelogenous leukemia (AML) [54]. In addition to cancer, PCPA has been reported to show pharmacological effects in α -herpes virus latent infection [41], globin disorders [47], metabolic disorders [48], and neurodegenerative disorders [55], suggesting that LSD1 inhibitors are useful as therapeutic agents for not only cancer but also non-cancerous diseases.

Ueda et al. designed LSD1-selective inhibitors on the basis of the structures of the methylated lysine substrate and PCPA (Fig. 4) [56]. PCPA-lysine analog hybrid compounds are expected to be potent LSD1-selective inhibitors because they can

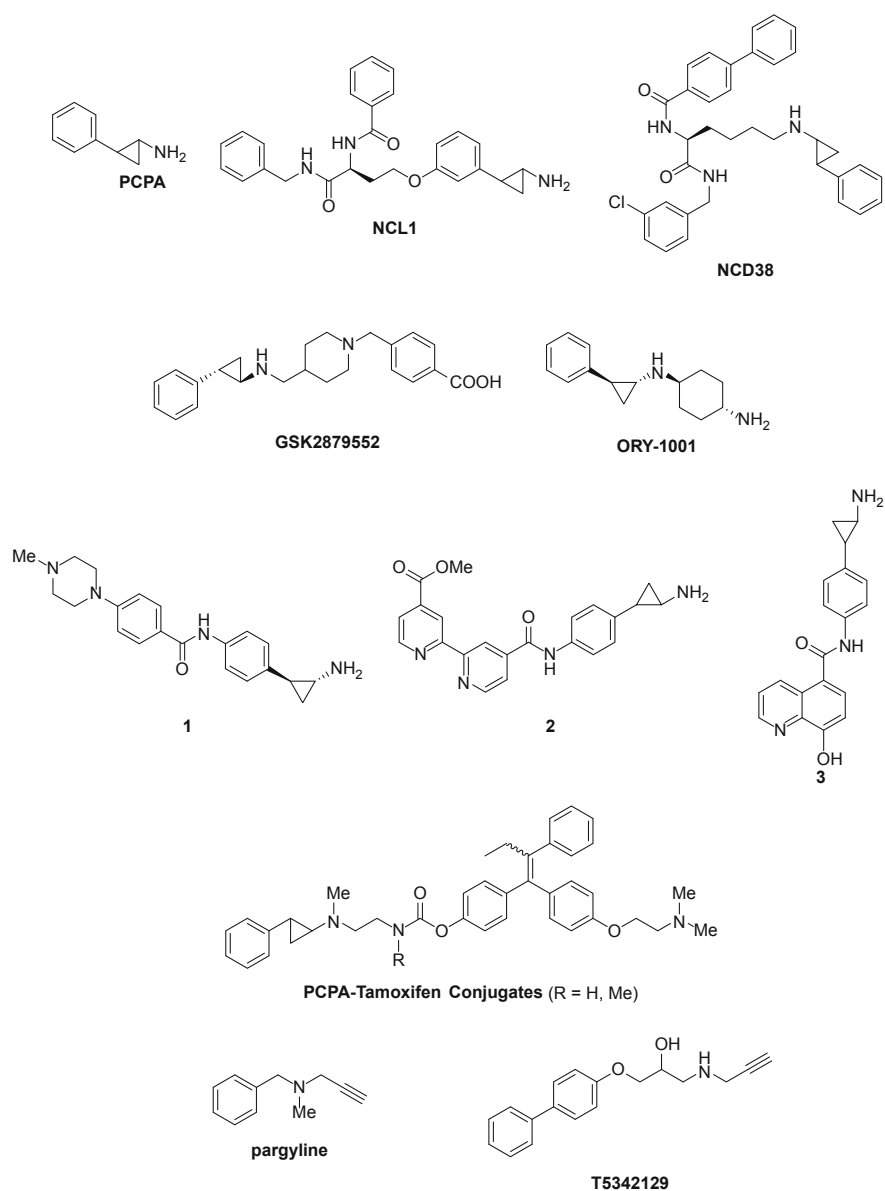


Fig. 2 Examples of LSD1 inhibitors

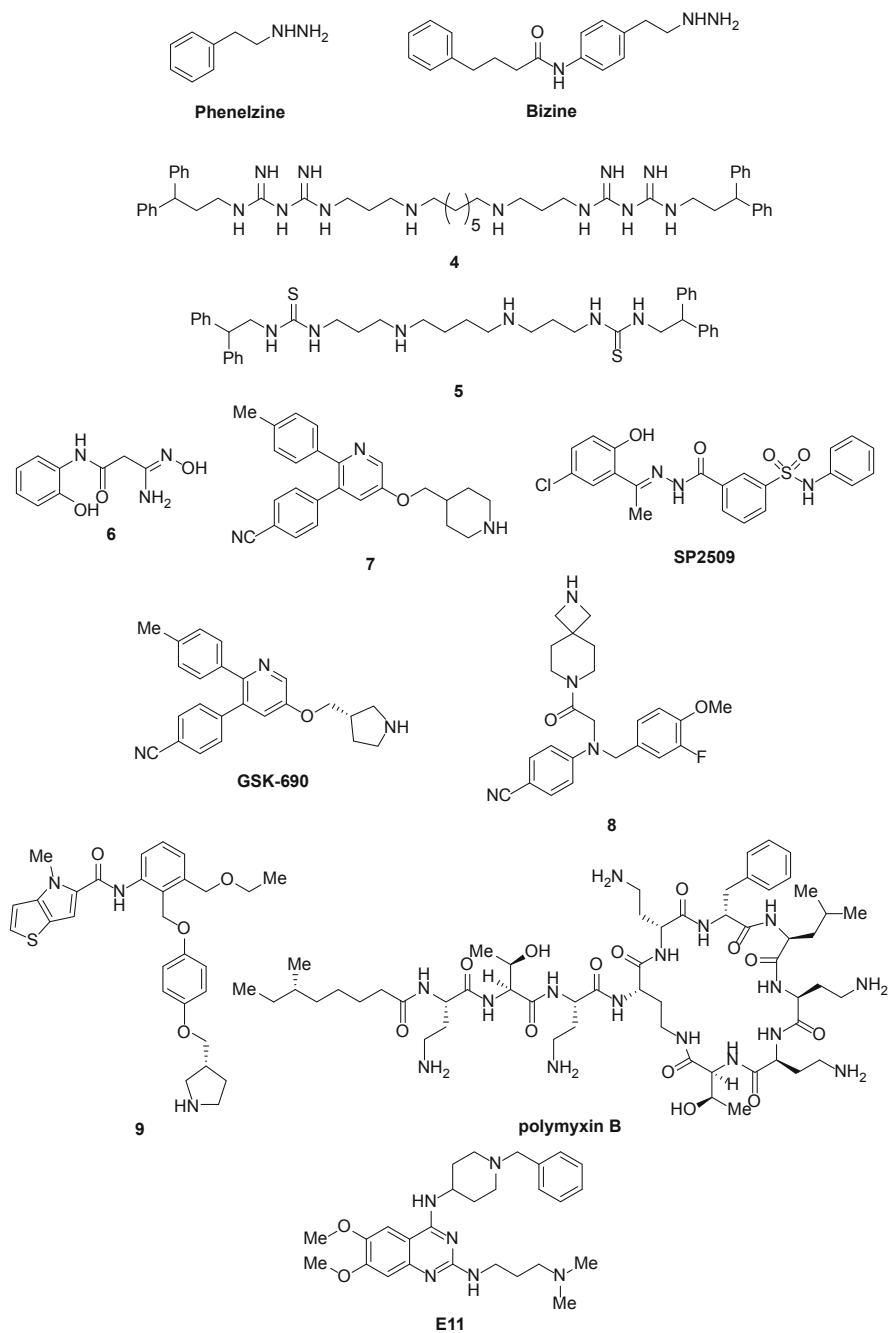


Fig. 2 (continued)

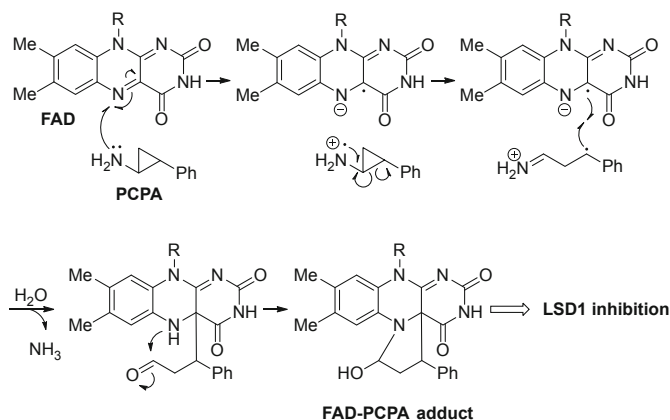


Fig. 3 Mechanism of LSD1 inhibition by PCPA

bind to the methylated lysine substrate-binding site of LSD1 and they possess the PCPA structure that reacts with FAD in the active site of LSD1. Small-molecule PCPA-lysine analog hybrid compounds were designed on the basis of the X-ray crystal structures of the FAD-PCPA adduct and the FAD-*N*-propargyl lysine peptide adduct in the active site of LSD1 [17, 52]. The superimposition of the two structures is shown in Fig. 4a. The FAD parts of the two adducts are well superimposed, and the phenyl ring of the FAD-PCPA adduct overlaps with ϵ -N and δ -C of the FAD-*N*-propargyl lysine peptide adduct. Based on these superimposed structures, PCPA-lysine analog hybrid compound NCL1 was designed (Figs. 2 and 4a), in which the side chain of the amino acid is linked to the phenyl ring of PCPA through an ether bond at the meta and para positions, respectively. Benzylamino and benzoyl groups were chosen as the substituents of the carbonyl and amino groups of the amino acid, respectively, because they were expected to be recognized by hydrophobic amino acid residues (Val 333, Ile 356, Phe 382, Leu 386, Leu 536, Ala 539, Thr 566, and Leu 677) at the entrance to the *N*-methylated lysine binding channel of LSD1 (Fig. 4b). In addition, the attachment of these small, hydrophobic groups could enhance membrane permeability. Furthermore, NCL1 was expected to selectively inhibit LSD1 over MAO-A and MAO-B, as the X-ray crystal structures of MAO-A and MAO-B indicated that their active-site cavities are not sufficiently capacious to accommodate the large group attached to the phenyl ring of PCPA in NCL1. NCL1 was prepared and its inhibitory activities toward human LSD1 and MAO-A and MAO-B were evaluated. Kinetic analysis and MS analysis suggested that the inhibitory activity of NCL1 occurs via the LSD1-directed synthesis of the FAD-PCPA adduct in the active site of LSD1 in a similar manner to PCPA (Fig. 3). As shown in Fig. 4c, NCL1 is a highly selective LSD1 inhibitor. Furthermore, NCL1 inhibits cancer cell growth at μM concentration, consistent with its effect on the methylation of H3K4, a substrate of LSD1. In addition, antiestrogen and NCL1 combination therapy suppresses the growth of drug-resistant breast cancer cells [57, 58]. NCL1 also reduces tumor volume in mice injected

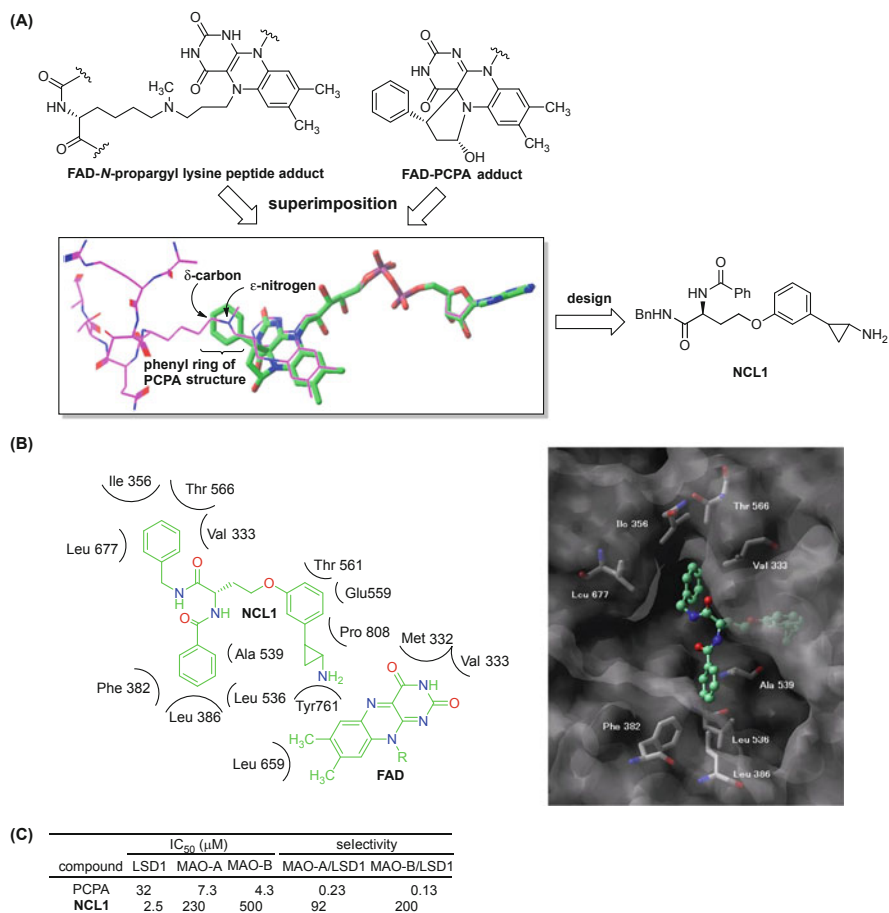


Fig. 4 (a) Design of an LSD1-selective inhibitor NCL1 based on superimposition of the FAD-PCPA adduct (PDB code: 2UXX) (tube) and the reduced FAD-*N*-propargyl lysine peptide adduct (PDB code: 2UXN) (wire) in the active site of LSD1. Amino acid residues in the active site are not shown for the sake of clarity. (b) View of the conformation of NCL1 (ball and stick) docked in the LSD1 catalytic core. Residues within 5 Å from NCL1 are displayed in the tube graphic. (c) In vitro LSD1, MAO-A, and MAO-B inhibitory activities of NCL1

subcutaneously with hormone-resistant prostate cancer PCa1 cells without adverse effects, suggesting the potential of LSD1 inhibitors as therapeutic agents for hormone-resistant prostate cancer [59]. These results point to the possibility of NCL1 as an anticancer agent.

Although NCL1 is a potent and selective LSD1 inhibitor, its activity in cell-based assays is insufficient. Ogasawara et al. had aimed to find novel LSD1 inactivators on the basis of the new concept “protein-targeted drug delivery” [60].

As mentioned above, PCPA inhibits LSD1 by forming a covalent bond with FAD (Figs. 3 and 5a). In the course of LSD1 inactivation by PCPA, the nitrogen atom of

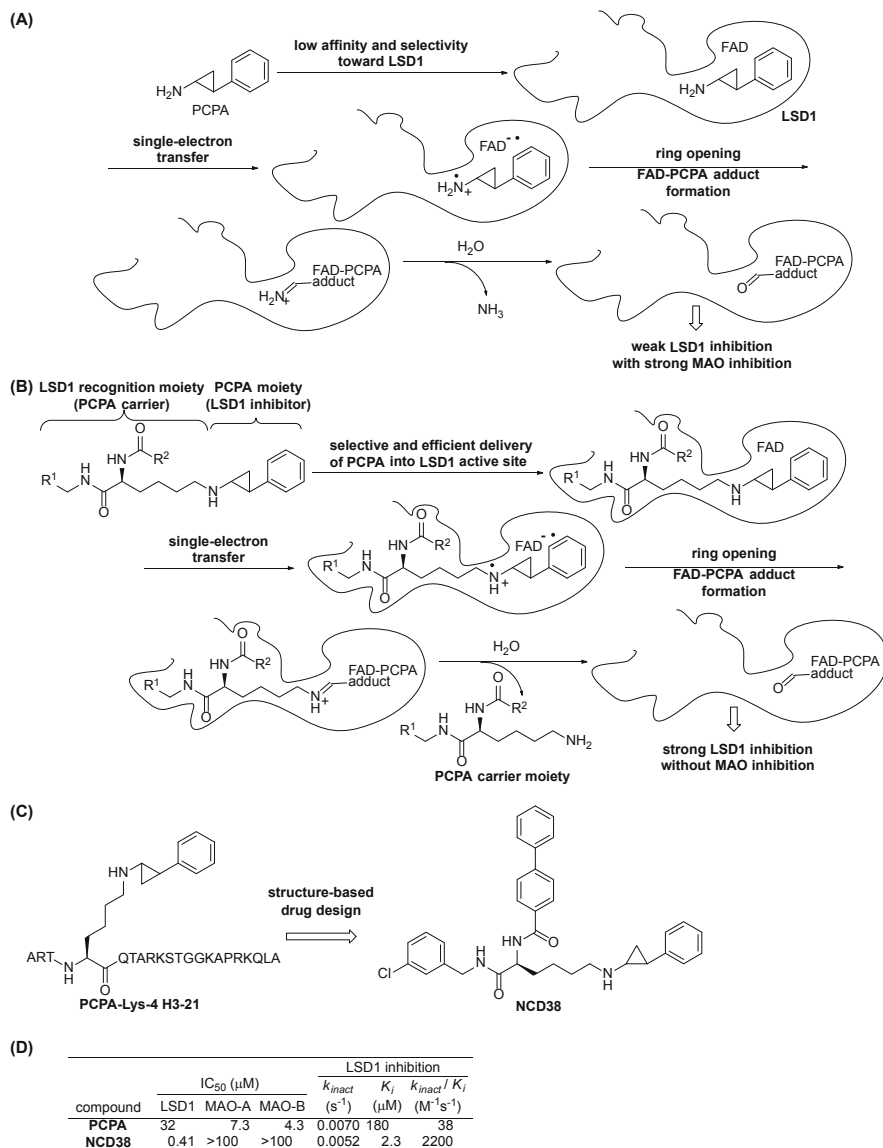


Fig. 5 (a) Mechanism of LSD1 inhibition by PCPA. (b) Mechanism of LSD1-targeted delivery of PCPA. (c) Structures of PCPA-Lys-4 H3-21 and NCD38. (d) LSD1 and MAO inhibitory activities and kinetic parameters of PCPA and NCD38

PCPA is released as an ammonia molecule through the hydrolysis of the imine intermediate. The nitrogen atom of the ammonia molecule corresponds to the ϵ -nitrogen atom of the lysine substrate in the proposed demethylation mechanism by LSD1 (Fig. 1). Taking these mechanisms into account, together with the idea of

delivering PCPA directly to the active site of LSD1, candidate LSD1 inactivators were designed (Fig. 5b), in which PCPA is coupled to a lysine carrier moiety at the nitrogen atom. Because methylated lysine is the substrate of LSD1, it is expected that the lysine moiety of the candidate inactivator would be efficiently recognized by LSD1, which would lead to high selectivity over MAO-A and MAO-B. After the PCPA moiety of the candidate inactivator is carried to the active site of LSD1, it is expected that the PCPA moiety would inactivate LSD1 in a similar manner to PCPA itself, namely, through single-electron transfer, radical opening of the cyclopropyl ring, and covalent bond formation with FAD (Fig. 5b). Then, the lysine moiety is expected to be released through the hydrolysis of the imine intermediate (Fig. 5b). Thus, the lysine moiety of the candidate inactivator serves as a carrier that delivers PCPA into the active site of LSD1 selectively and efficiently.

Initially, as a proof of concept study, PCPA-Lys-4 H3-21 (Fig. 5c), which bears a PCPA moiety at Lys-4 of a 21-amino-acid LSD1 substrate peptide (H3-21), was designed and prepared. As expected, PCPA-Lys-4 H3-21 strongly inhibited LSD1 with an IC_{50} of 0.16 μ M in a time- and concentration-dependent manner, but did not inhibit MAO-A or MAO-B ($IC_{50} > 100 \mu$ M). However, PCPA-Lys-4 H3-21 showed only weak antiproliferative activity in cancer cells where LSD1 was overexpressed. It was speculated that PCPA-Lys-4 H3-21 has poor membrane permeability, likely as a result of the high polarity of its peptide structure. Thus, based on this proof of concept of the LSD1-targeted PCPA delivery strategy, this strategy was applied to the design of nonpeptide, small-molecule LSD1 inactivators that show activity in cell-based assays. A number of candidate small-molecule, drug-delivery-type LSD1 inactivators were designed and synthesized guided by the X-ray crystal structure of LSD1, and NCD38 was eventually identified as a potent and selective LSD1 inactivator (Figs. 2 and 5c). NCD38 also showed potent antiproliferative activity in solid cancer cells.

In addition, the LSD1 inactivation mechanism was investigated to confirm that NCD38 indeed inhibits LSD1 by delivering PCPA to the LSD1 active site (Fig. 5b). Kinetic analysis revealed that NCD38 is a time-dependent LSD1 inactivator, in accordance with the irreversible mechanism we proposed (Fig. 5b). The kinetic parameters of NCD38 are shown in Fig. 5d. The k_{inact}/K_i value of NCD38 is much larger than that of PCPA, thus confirming that NCD38 is a much more potent LSD1 inactivator than PCPA. MALDI MS analysis of the inactivated mixture of LSD1 with NCD38 was also performed. Peaks with m/z 918 and 900, corresponding to the FAD-PCPA adduct and the dehydrated adduct, respectively, were observed in the mixture of LSD1/NCD38. The lysine moiety released from LSD1/NCD38 was also detected. These mechanistic data strongly support the idea that NCD38 inhibits LSD1 through the efficient and selective delivery of PCPA to the active site of LSD1 with the assistance of its lysine moiety (Fig. 5b).

Interestingly, a recent report has shown that NCD38 derivatives inhibit LSD1 in preference to LSD2 [61].

Recently, it has been reported that NCD38 inhibits the growth of MLL-AF9 leukemia as well as erythroleukemia, megakaryoblastic leukemia, and myelodysplastic syndrome (MDS) overt leukemia cells in the concentration range in which normal

hematopoiesis is spared [62]. A single administration of NCD38 causes the *in vivo* eradication of primary MDS-related leukemia cells with a complex karyotype. Mechanistic studies showed that NCD38 elevates H3K27ac level on enhancers of the LSD1 signature genes and derepresses the super-enhancers of hematopoietic regulators that are silenced abnormally by LSD1.

In addition to the antiproliferative activity of NCD38 in differentiated cancer cells, NCD38 is also able to inhibit cancer stem cell formation and the maintenance of human metastatic breast cancer cells, thus reverting them to epithelial form [63]. The pharmacological inhibition of LSD1 using NCD38 significantly reduces cell viability and neurosphere formation and induces apoptosis of glioma stem cells (GSCs) with little effect on differentiated cells [64]. In preclinical studies using orthotopic models, NCD38 significantly reduces GSC-driven tumor progression and improves mouse survival. Mechanistic studies showed that NCD38 causes apoptosis of GSCs by inducing the activation of the unfolded protein response pathway.

Thus, NCD38 and its derivatives are considered candidates for anticancer agents as well as tools for probing the biology of LSD1. Currently, IMG-7289, an NCD38 mimetic, is being evaluated in a phase 1/2 clinical trial for the treatment of AML and MDS (<http://www.imagobio.com/imago-biosciences-doses-first-patients-in-phase-1-2-study-of-img-7289-in-myelofibrosis/>).

GlaxoSmithKline and Oryzon Genomics discovered potent and selective LSD1 inhibitors GSK2879552 and ORY-1001, respectively (Fig. 2) [65, 66]. GSK2879552 and ORY-1001 exhibit antileukemia activity and are currently undergoing clinical trials for AML treatment. In addition, screening for cancer cell lines revealed that small cell lung carcinoma (SCLC) is sensitive to LSD1 inhibition by PCPA analogs, including GSK2879552 [65]. GSK2879552 exhibits DNA hypomethylation in SCLC lines, suggesting that DNA hypomethylation can be used as a predictive biomarker of LSD1 inhibitory activity.

In addition, Oryzon Genomics developed LSD1/MAO-B inhibitor ORY-2001. ORY-2001 prevents the development of memory deficit in SAMP8 mice through the induction of neuronal plasticity and the reduction of neuroinflammation [49]. Currently, ORY-2001 is being evaluated in a phase 1 clinical trial for the treatment of Alzheimer's disease.

Vianello et al. reported a novel PCPA derivative **1** (Fig. 2) as a potent inhibitor of LSD1 [67]. PCPA derivative **1** strongly inhibits the clonogenic potential of acute leukemia cell lines. Furthermore, compound **1** exhibits *in vivo* efficacy after oral administration, inducing a 62% increase in survival in a mouse leukemia model.

Mai and co-workers identified hybrid LSD1/JmJc-domain containing histone lysine demethylase inhibitors by coupling the skeleton of PCPA, an LSD1 inhibitor, with 4-carboxy-4-carbomethoxy-2,2-bipyridine or 5-carboxy-8-hydroxyquinoline, two 2-oxoglutarate competitive templates developed for JmJc inhibition [68]. The hybrid compounds were validated as potential antitumor agents in cells. Compounds **2** and **3** (Fig. 2) caused growth arrest and substantial apoptosis in LNCaP prostate and HCT116 colon cancer cells with an increase in H3K4 and H3K9 methylation levels in the cells.

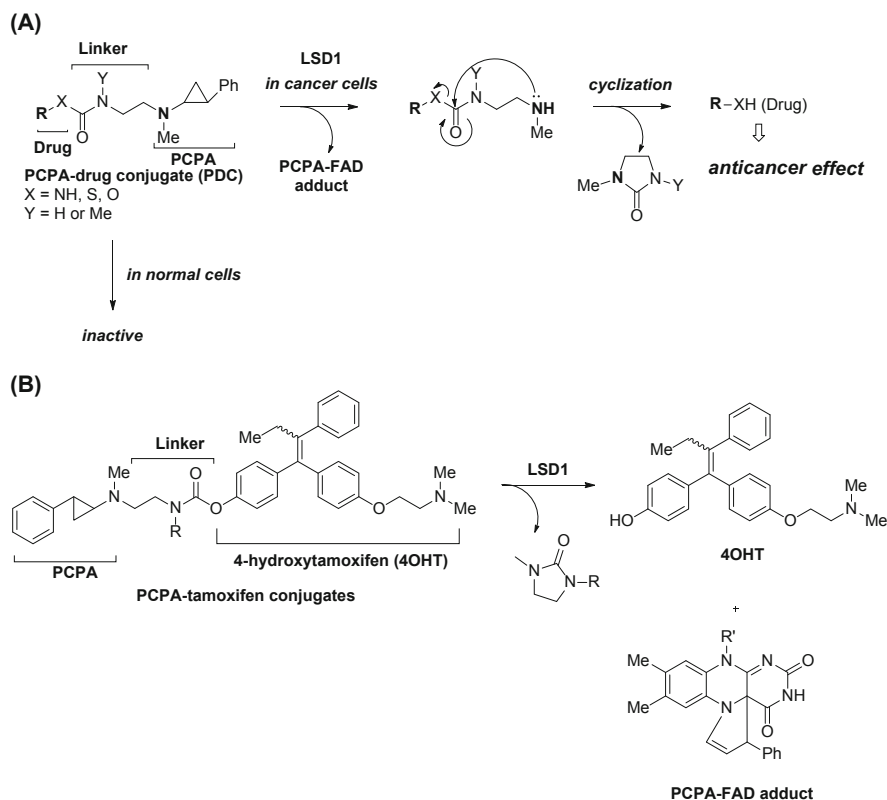


Fig. 6 (a) Concept of small-molecule-based drug delivery system for cancer therapy using PCPA-drug conjugates (PDCs). (b) Release of 4-hydroxytamoxifen (4OHT) from PCPA-tamoxifen conjugates in the presence of LSD1

The chemotherapy of cancer, including targeted therapy using anticancer drugs, usually provides a certain level of beneficial therapeutic effect while simultaneously causing serious adverse effects on account of the cytotoxicity of the employed drugs toward normal cells. In order to reduce the adverse effects of anticancer drugs, several drug delivery systems (DDSs) for anticancer drugs have been developed. One example is antibody-drug conjugates (ADCs) that show both potent and selective cytotoxicity toward cancer cells that express a specific antigen. Some of these ADCs, such as brentuximab vedotin and trastuzumab emtansine, are currently used in clinical practice. However, because of their macromolecular structure, ADCs have several limitations, including poor tissue penetration, immunogenicity, low bioavailability, and high cost. To overcome the problems of macromolecule-based DDSs, such as ADCs, Ota et al. focused on LSD1 to trigger the controlled release of anticancer drugs in cancer cells where LSD1 is highly expressed [69]. For that purpose, conjugates of the LSD1 inhibitor PCPA are used as novel anticancer drug

delivery molecules to selectively release anticancer drugs through the inhibition of LSD1 (Fig. 6a). PCPA-drug conjugates (PDCs) are expected to be recognized by LSD1 and to inactivate it in a similar manner to PCPA itself, i.e., via a single-electron transfer mechanism (Fig. 6b). Subsequently, the drug should be released together with the linker moiety of the PDCs through hydrolysis of the imine intermediate, and an ensuing intramolecular cyclization should eventually separate the linker from the drug. Thus, PDCs could serve as delivery molecules that selectively release an anticancer drug upon binding to LSD1. This method would significantly reduce adverse effects as such molecules are inactive toward normal cells where the expression of LSD1 is low. As a PDC prototype, we have designed and synthesized PCPA-tamoxifen conjugates (Fig. 2) targeting breast cancer cells (Fig. 6b), which release 4-hydroxytamoxifen in the presence of LSD1 in *in vitro* assays. Furthermore, PCPA-tamoxifen conjugates inhibit the growth of breast cancer cells through the simultaneous inhibition of LSD1 and ER α without exhibiting cytotoxicity toward normal cells. These results demonstrated that PDCs are a useful anticancer drug delivery tool that may facilitate the selective release of drugs in cancer cells.

Previously, Schüle and co-workers tested whether pargyline (Fig. 2), a well-known MAO inhibitor, inhibits LSD1 and found that it blocks the demethylation of H3K9 by LSD1 and consequently blocks AR-dependent transcription [7]. Based on this report, Jung and co-workers discovered new small-molecule inhibitors of LSD1 containing a propargylamine warhead [70]. Druglike LSD1 inhibitors with a propargylamine moiety, such as T5342129 (Fig. 2), showed histone hypermethylation in breast cancer cells.

Phenelzine (Fig. 2), a MAO inhibitor, has also been reported to inhibit LSD1, although its inhibitory activity and selectivity for LSD1 are very low [51, 52, 71, 72]. Cole and co-workers identified analogs of phenelzine and their LSD1 inhibitory properties [73]. A novel phenelzine analog (bizine) (Fig. 2) containing a phenylbutyrylamide appendage was shown to be a potent LSD1 inhibitor *in vitro* and was selective versus MAO-A, MAO-B, and LSD2. Bizine was found to be effective in modulating bulk histone methylation in cancer cells, and ChIP-seq experiments revealed a statistically significant overlap in the H3K4 methylation pattern of genes affected by bizine and those altered in LSD1^{-/-} cells. Treatment of two cancer cell lines, LNCaP and H460, with bizine resulted in a reduction of proliferation rate, and bizine showed additive to synergistic effects on cell growth when used in combination with two of the five HDAC inhibitors tested. Moreover, neurons exposed to oxidative stress were protected by the presence of bizine, suggesting potential applications in neurodegenerative diseases.

Not only irreversible LSD1 inhibitors, which are derived from MAO inhibitors, but also reversible LSD1 inhibitors have been identified.

Woster and co-workers reported that (bis)guanidines, (bis)biguanides, and their urea and thiourea isosteres, such as **4** and **5** (Fig. 2), are potent inhibitors of LSD1 and induce the re-expression of aberrantly silenced tumor suppressor genes in tumor

cells in vitro [31]. They also reported a series of small-molecule amidoximes, such as **6** (Fig. 2), which are moderate inhibitors of recombinant LSD1 but produce dramatic changes in methylation at the H3K4 chromatin mark in Calu-6 lung carcinoma cells [74]. In addition, these analogs increased cellular levels of LSD1 target genes, such as secreted frizzled-related protein 2, H-cadherin, and the transcription factor GATA4.

Wu et al. reported 3-(piperidin-4-ylmethoxy)pyridine-containing compounds, such as **7** ($K_i = 29$ nM) (Fig. 2), as potent LSD1 inhibitors [75]. These compounds exhibit high selectivity ($>160\times$) against related MAO-A and MAO-B. Enzyme kinetics and docking studies suggested that they are competitive inhibitors against a dimethylated H3K4 substrate and proposed a possible binding mode. The potent LSD1 inhibitors can increase cellular H3K4 methylation and strongly inhibit the proliferation of several leukemia and solid tumor cells with EC_{50} values as low as 280 nM, while they have negligible effects on normal cells.

A structure-based virtual screening of a compound library containing approximately two million small molecular entities has led to the identification of SP2509 (Fig. 2) as a reversible and selective LSD1 inhibitor ($K_i = 31$ nM) [76], although the core *N'*-(2-hydroxybenzylidene)hydrazide motif was previously identified as a pan-assay interference compound [77]. SP2509 inhibits the proliferation and survival of several cancer cell lines, including breast and colorectal cancer. It was also reported that treatment with SP2509 attenuates the binding of LSD1 with the corepressor CoREST, increases the permissive H3K4Me3 mark on the target gene promoters, and increases the levels of p21, p27, and CCAAT/enhancer binding protein α in cultured AML cells [78]. In addition, SP2509 inhibits colony growth of AML cells. SP2509 also induces morphological features of differentiation in cultured and primary AML blasts. Treatment with SP2509 alone significantly improves the survival of immune-depleted mice following tail-vein infusion and engraftment of cultured or primary human AML cells. Co-treatment of panobinostat, a pan-HDAC inhibitor, and SP2509 synergistically improves survival in mice engrafted with the human AML cells without exhibiting any toxicity.

Recently, Mould et al. identified reversible LSD1 inhibitors from a high-throughput screen and subsequent in silico modelling approaches [79]. Based on a hit compound, they carried out scaffold hopping from GSK-690 (Fig. 2) [80, 81], an analog of compound **7**, to find compound **8** (Fig. 2), which has a K_d value of 32 nM and an EC_{50} value of 0.67 μ M, in a surrogate cellular biomarker assay. Compound **8** does not display the same level of hERG liability as that observed with GSK-690 and represents a promising lead for the further development of LSD1 inhibitors.

In addition, Sartori, Vianello, and co-workers discovered thieno[3,2-*b*]pyrrole-5-carboxamide analog **9** (Fig. 2) as a potent reversible inhibitor of LSD1 [82, 83]. Compound **9** upregulated the expression of LSD1 target genes such as CD14, CD11b, and CD86 in THP-1 cells, and it showed a remarkable anticolonogenic cell growth effect on MLL-AF9 human leukemia cells.

It was also reported that polymyxins and the antibiotics polymyxins such as polymyxin B and quinazolines such as E11 (Fig. 2) inhibit LSD1 by binding to the entrance of the substrate cleft where their positively charged moiety interacts

with anionic amino acid residues of LSD1 [84]. These scaffolds should be useful for further studies on LSD1 inhibitor development.

6 Summary

At present, there are only six approved epigenetic drugs (two DNMT inhibitors and four HDAC inhibitors), and they are utilized only for MDS, cutaneous T-cell lymphoma, peripheral T-cell lymphoma, or multiple myeloma treatment. Additional indications of the current epigenetic drugs are limited. In this regard, researchers need to acquire an integrated understanding of cancer epigenetics in order to discover useful next-generation drugs for cancer therapy.

In this chapter, the biology and pharmacology of LSD and hitherto reported LSD inhibitors have been presented. In particular, small-molecule LSD1 inhibitors have been discussed from the point of view of potential therapeutic agents. It is hoped that the LSD1-selective inhibitors presented here will provide the basis for the development of novel therapeutic agents for both cancer and non-cancerous diseases.

Compliance with Ethical Standards

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