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PERSPECTIVE

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Lysine Demethylases Inhibitors

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1. INTRODUCTION

Recent studies have revealed that chromatin remodeling, caused by DNA methylation and histone modifications such as acetylation, methylation, and phosphorylation, plays a pivotal role in DNA replication/repair and the regulation of epigenetic gene expression.[1−6] Among the posttranscriptional histone modifications, lysine methylation is one of the most widely studied, and methylation at various sites, including lysine 26 of histone 1 (H1K26), H3K4, H3K9, H3K27, H3K36, H3K79, and H4K20, has been shown to lead to transcriptional activation or silencing.[7] In general, methylation at H3K4 is associated with actively transcribed gene loci, whereas methylation at H3K9 and H3K27 leads to transcriptional silencing.[8] However, the situation is further complicated by the fact that the ε-amino group of lysine residues can be mono-, di-, or trimethylated, and differential methylation at each lysine methylation site provides functional diversity. For example, dimethylation at H3K4 is associated with both inactive and active genes, whereas trimethylation is exclusive to active genes.[9]

In contrast to other histone modifications, such as acetylation and phosphorylation, histone lysine methylation had been regarded as irreversible because of the high thermodynamic stability of the N−C bond. Indeed, while a number of histone lysine methyltransferases (HKMTs) had been identified by 2003,[7] histone lysine demethylases (KDMs) had not been identified. However, two classes of KDMs have been identified since 2004. One class includes lysine-specific demethylase 1 (LSD1, also known as KDM1A) and LSD2 (also known as KDM1B), which are flavin-dependent amine oxidase domain-containing enzymes.[10,11] The other class comprises the recently discovered Jumonji domain-containing protein (JMJD) histone demethylases,[12,13] which are Fe(II) and α-ketoglutarate-dependent enzymes. The identification of these KDMs established that histone methylation is reversibly regulated by HKMTs and KDMs.

As there is increasing evidence that KDMs are associated with various disease states,[14] they have emerged as attractive targets for the development of new therapeutic drugs. To date, several classes of KDM inhibitors have been identified. In this Perspective, we review the reported KDM inhibitors and discuss their potential as therapeutic agents.

2. BIOLOGY AND PHARMACOLOGY OF FLAVIN-DEPENDENT LYSINE DEMETHYLASES

LSD1, the first histone demethylase to have been discovered, removes the methyl groups from mono- and dimethylated Lys4 of histone H3 (H3K4me1/2) through flavin adenine dinucleotide (FAD) dependent enzymatic oxidation.[10] In prostate cell lines, LSD1 also demethylates H3K9me1/2 and regulates androgen receptor-mediated transcription.[15] The targets of LSD1 regulatory demethylation are not limited to histone H3; LSD1 also demethylates p53,[16] DNA methyltransferase 1,[17] STAT3,[18] E2F1,[19] and MYPT1[20] and regulates their cellular functions.

It has been reported that LSD1 binds with several transcription factors and regulates the expression of a number of genes (Table 1). For example, the LSD1-RE1-silencing transcription factor corepressor (CoREST)−histone deacetylase 1 (HDAC1) complex demethylates H3K4me2 and me1 and decreases the expression of REST-responsive genes such as synapsin (*SYN*) and muscarinic acetylcholine receptor 4 (*MuAchR4*) in HEK293 cells.[21]

LSD1 is overexpressed in various cancer cells and tissues: neuroblastoma,[38] retinoblastoma,[26] prostate cancer,[15,35] breast cancer,[31,39,40] lung cancer,[41] and bladder cancer cells.[41] Furthermore, the outcome of RNAi-mediated knockdown or inhibition of LSD1 suggested that this enzyme is associated with cancer cell growth by modulating prosurvival gene expression and p53 transcriptional activity.[31,38,42,43] Therefore, LSD1 inhibitors are of interest not only as tools for elucidating in detail the biological functions of the enzyme but also as potential anticancer agents.

LSD1 also regulates viral gene transcription.[44] In herpes simplex viruses (HSV) and varicella zoster viruses (VZV), increase in methylation of H3K4 and decrease in methylation of H3K9 are needed for viral gene transcription in a host cell.[45] To increase methylation, the virus recruits host cell factor 1 (HCF-1) and an HKMT complex. Kristie and co-workers showed that LSD 1 interacts with the HCF-1 component of the HKMT complex and demethylates H3K9.[44] They also showed that blocking LSD1 activity led to inhibition of viral gene transcription. These results suggested that LSD1 inhibitors could work as anti-HSV and anti-VZV agents.

LSD2, the other flavin-dependent lysine demethylase, was identified in 2009,[11] and relatively little is yet known about it. It has been reported that H3K4 demethylation by LSD2 establishes the DNA methylation imprints during oogenesis[46] and activates transcription,[47] while it was also reported that LSD2 represses transcription and the repression activity is independent of its demethylase activity.[48]

3. STRUCTURAL STUDIES AND CATALYTIC MECHANISM OF FLAVIN-DEPENDENT LYSINE DEMETHYLASES

The X-ray crystal structure of LSD1 complexed with CoREST and a histone H3 peptide was determined by Yang et al.[49] This

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Table 1. Genes Regulated by LSD1

regulation	LSD1 target genes	LSD1-binding proteins	refs
repression	*SYN1, MuAchR4, SCN1A, SCN2A, SCN3A, SCG10*	REST, CoREST, HDAC1, HDAC2	10, 21−23
	GFI-1b, C-MYB, NM_026543	Gfi-1, Gfi-1b, CoREST, HDAC1, HDAC2	24
	Gh	ZEB1, CoREST, HDAC1, HDAC2, LCoR, PC2	25
	PTEN	TLX, CoREST, HDAC1, HDAC2	26
	P4.2	TAL1, CoREST, HDAC1, HDAC2	27
	CIITA	Blimp-1, HDAC1, HDAC2	28
	RARβ2, CYP26, p21 WAF1, HOXa1	ASXL1, RAR, HP1	29
	E-cadherine, CLDN7, KRT8	Snai1, CoREST, HDAC1	30
	TESC, cyclin A1, CSRP2, ADAMTS1, PSMB9	not identified	31 10, 21−23
	SFRPs, GATAs	not identified	32
	Ifi202, Ifi204, Hes1, Notch1, HoxA9, HoxA 10:1, HoxA 10:2, Hey1, Hey2, Gata3	SIRT1, CoREST, CtBP1	33
	dpp	not identified	34
activation	*Gh, PRL, TSHB, PITI*	PIT1, WDR5	25
	PSA	AR, KDM4C	15, 35
	pS2, GREB1	ERα	36
	Cad, Ncl	Myc, OGG1, Ape1	36, 37
	S100A8, PLCL1, LEPR, DR1, DEK	not identified	31

crystal structure 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 comprising Asn, Trp, and two Asp residues (Figure 1). The structure also confirmed the positioning of the lysine methyl groups in sufficient proximity to FAD for FAD-mediated catalysis.

The crystal structures of LSD1 and detailed analysis of the catalytic mechanism have led to a solid understanding of the catalytic mechanism of demethylation of methylated lysine substrates (Scheme 1).[10,49−51] First, the methylated lysine substrate is converted to an iminium cation, presumably through a two single-electron oxidation of the amine by FAD. Next, the addition of a water molecule to the iminium cation and subsequent deformylation afford demethylated lysine. The $FADH_2$ 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, 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.

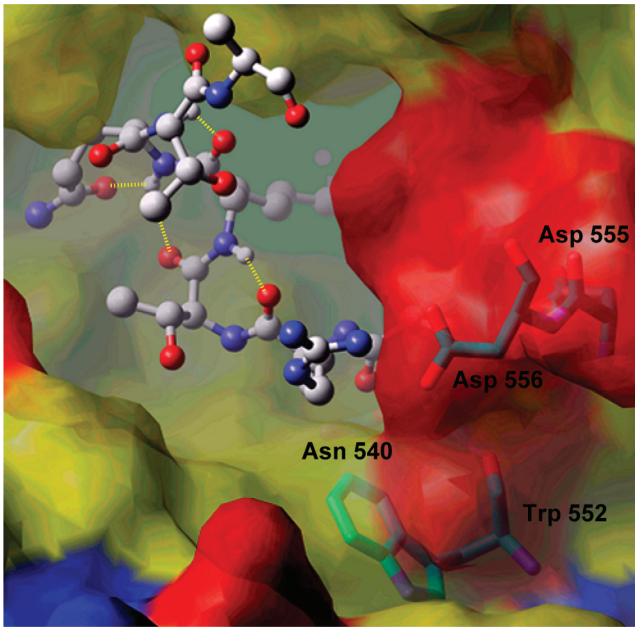

Figure 1. X-ray crystal structure of LSD1 complexed with CoREST and a histone H3 peptide (PDB code 2UXN). Amino acid residues of LSD1 and the histone H3 peptide are displayed as tube and ball-and-stick models, respectively.

4. FLAVIN-DEPENDENT LYSINE DEMETHYLASE INHIBITORS

As mentioned above, 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 (17.6% identity).[52,53] Therefore, MAO inhibitors might inhibit LSD1. Schüle and co-workers tested whether pargyline (**1**) (Figure 2), a well-known monoamine oxidase inhibitor,

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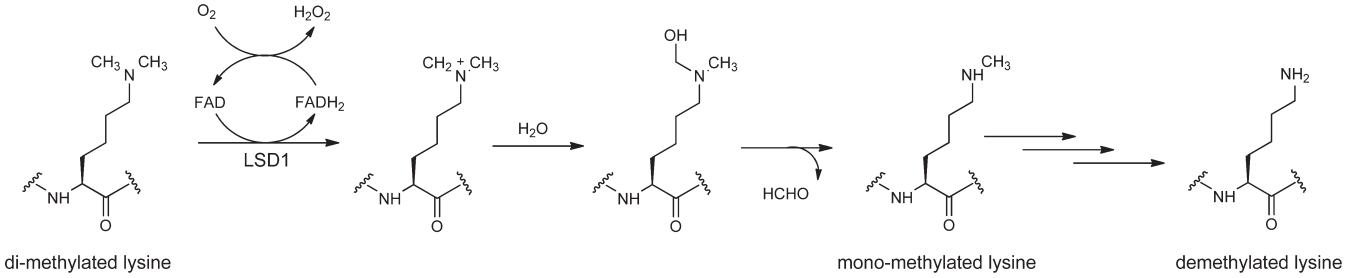
Scheme 1



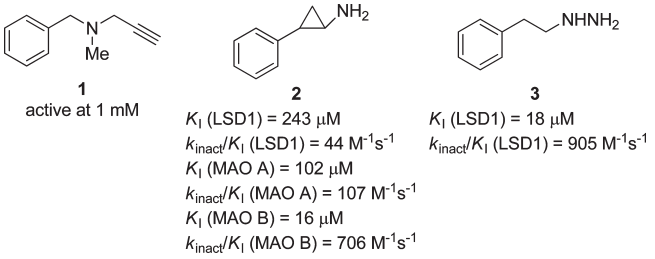


Figure 2. MAO inhibitors that inhibit LSD1.

inhibits LSD1 and found it blocks demethylation of H3K9 by LSD1 and consequently blocks androgen receptor-dependent transcription.[15] Other MAO inhibitors such as *trans*-2-phenylcyclopropylamine (PCPA, **2**) and phenelzine (**3**) (Figure 2) have been reported to inhibit LSD1, although their inhibitory activity and selectivity for LSD1 are very low.[52,54−56]

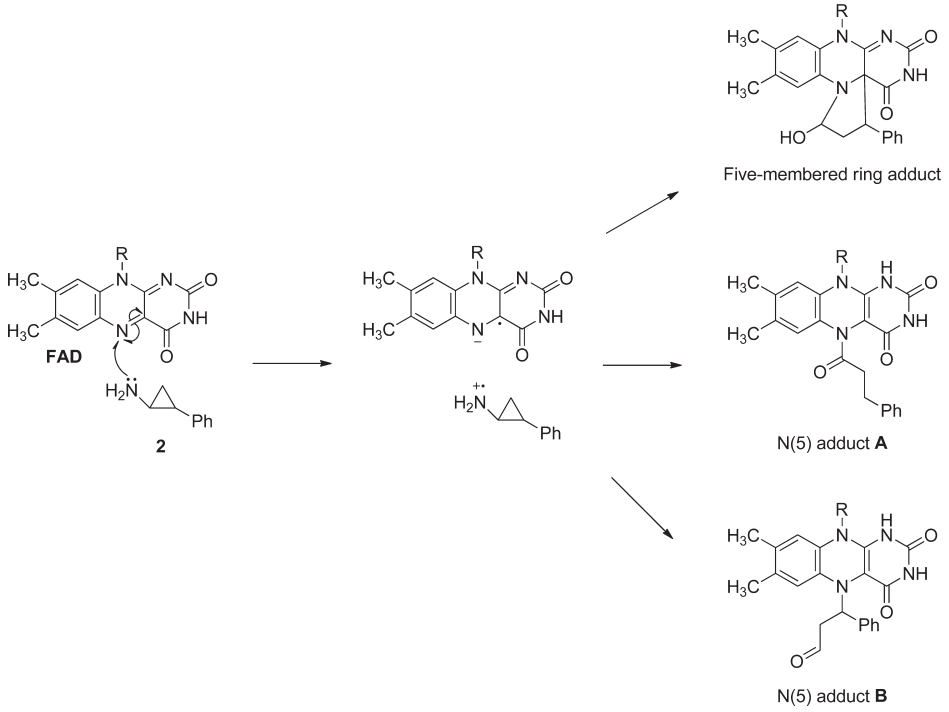
Among MAO inhibitors, **2** is the best studied LSD1 inhibitor. Schmidt and McCafferty demonstrated that **2** is a mechanism-based irreversible inhibitor of LSD1, based on kinetics and MS analysis.[52] LSD1 inhibition by **2** occurs via formation of a covalent adduct with the flavin ring following one-electron oxidation and cyclopropyl ring-opening (Scheme 2). Three different types of **2**−FAD adduct have been suggested based on X-ray crystal structure analyses of LSD1 complexed with **2**. Yu and co-workers showed that the structure of **2**−FAD complex in LSD1 is a five-membered ring adduct.[55] On the other hand, Yokoyama and co-workers suggested that the **2**−FAD complex is not completely composed of the five-membered ring adduct but partially contains an intermediate such as N(5) adduct **A**.[57] In addition, structural analyses of LSD1 complexed with chiral PCPAs and its derivatives implied that (1*R*,2*S*)-**2** reacts with FAD in the active site to yield N(5) adduct **A**, whereas (1*S*,2*R*)-**2** generates another N(5) adduct **B**.[58]

Although the LSD1-inhibitory activity of MAO inhibitors is weak, high concentrations (>0.1 mM) of **2** induced an increase of global H3K4 methylation and growth inhibition of neuroblastoma cell and bladder cancer cells.[38,59] Furthermore, ip injection of 2 mg of **2** once daily for 21 days reduced neuroblastoma xenograft growth in mouse models.[38] As well as having anticancer activity, **2** was found to have an antiviral effect. Kristie's group treated HSV- or VZV-infected cells with high concentrations (>1 mM) of **2** and observed dose-dependent decreases in viral immediate−early mRNA and proteins.[44] **2** also lowered the amount of HSV by nearly 3 orders of magnitude compared with vehicle.

Derivatives of **2** have been reported to inhibit LSD1 more potently and selectively than PCPA itself. Gooden et al. efficiently synthesized several substituted PCPAs and examined their inhibitory activity toward LSD1, MAO A, and MAO B; they found that compound **4** (Figure 3) is a more potent and selective LSD1 inhibitor than **2**.[60] Ganesan's group prepared optically active PCPA analogues and identified (1*R*,2*S*)-4-bromo-PCPA (**5**) (Figure 3) as an LSD1 inhibitor more potent than **2** in both LSD1 inhibition assays and human prostate LNCaP cell growth inhibition assays.[61] NCL1 (**6**) and NCL2 (**7**) (Figure 3), which are lysine−PCPA hybrid compounds designed based on the crystal data, were discovered as the first cell-active LSD1-selective inhibitors.[42] These small molecule PCPA−lysine analogues showed LSD1 selectivity that was 400−11000 times greater than that of **2**. In human cancer cell lines, the compounds inhibited cell growth at 6−67 μM, above which distinct H3K4 methylation was detected. In addition, it was recently reported that (1*S*,2*R*)-NCL1 is approximately 4 times more potent than (1*R*,2*S*)-NCL1 in enzyme assays.[62] Since the discovery of **6** and **7**, several potent PCPA-based LSD1 inhibitors have been identified. Compound **8** (Figure 3) showed selective inhibitory activity for LSD1 and MAO A over LSD2 and MAO B.[58] In a cellular model of acute promyelocytic leukemia, compound **8** caused cell growth inhibition and acted synergistically with retinoic acid, an antileukemia drug. Structure-based drug design led to the identification of S2101 (**9**) (Figure 3), which shows potent LSD1 inhibition and selectivity for LSD1 over MAO A and MAO B.[63] Compound **9** increased H3K4me2 levels in HEK293T cells at a concentration as low as 1 μM. Recently, N-alkylated PCPA analogues such as **10** and **11** (Figure 3) have been reported.[64−66] These compounds inhibited LSD1 with high potency and selectivity over MAO A and MAO B, although full details have not been disclosed.

Propargyl-Lys-4 H3-21 peptide (**12**) and hydrazine-Lys-4 H3-21 peptide (**13**) (Figure 4), which were designed based on the structures of **1** and **3**, respectively, have been reported to be LSD1-selective inhibitors.[49,56,67,68] The mechanism of LSD1 inhibition by peptide **12** involves conjugate addition of the flavin N5 to the γ carbon of the electrophile following two-electron oxidation to the iminium ion (Scheme 3). The propargyl lysine peptide **12** is selective for LSD1 over MAO-B and can be used as a biochemical tool for in vitro study of LSD1. Peptide **13** is an LSD1 inhibitor 20-fold more potent than peptide **12**. The mechanism of LSD1 inhibition by peptide **13** has been suggested to be as shown in Scheme 3. The LSD1 inhibition mechanism by peptide **12** initially involves a two-electron oxidation to form the corresponding diazene. After reoxidation of the FAD by molecular oxygen, two-electron oxidation of the diazene yields the diazonium species, an excellent leaving group. Attack from N(5) of the reduced flavin leads to the peptide−FAD adduct with loss of N_2.

Scheme 2



In addition to the mechanism-based LSD1 inhibitors mentioned above, polyamine-based LSD1 inhibitors, which were inspired by the homology between LSD1 and FAD-dependent polyamine oxidases, have been reported.[32,43,69] Huang et al. demonstrated that (bis)biguanide analogues such as **14** (Figure 5) inhibit LSD1 noncompetitively at <2.5 μM and inhibition of LSD1 by **14** increased H3K4me2, leading to re-expression of aberrantly silenced genes important in the development of colon cancer, including members of the secreted frizzle-related proteins (*SFRPs*) and the *GATA* family of transcription factors.[32] Woster and co-workers reported that a series of isosteric ureas and thioureas such as **15** (Figure 5) also inhibit LSD1, induce increased methylation at H3K4, and increase *SFRP2* and *GATA4* mRNA in Calu-6 lung carcinoma cells. Further, thioureas including **15** showed Calu-6 cell growth inhibition with GI_{50} of 9−40 μM.[69] More importantly, Huang et al. showed that co-treatment with oligoamine LSD1 inhibitor **16** (Figure 5) and a DNA methyltransferase inhibitor results in significant inhibition of the growth of established tumors in a human colon cancer tumor model in vivo.[43]

5. BIOLOGY AND PHARMACOLOGY OF JUMONJI C-CONTAINING LYSINE DEMETHYLASES

JMJD histone demethylases remove the methyl groups from methylated histone lysines through Fe(II)/α-ketoglutarate-dependent enzymatic oxidation.[12,13] To date, a number of JMJD histone demethylases have been identified, and they display substrate specificity (Table 2). While demethylation by LSD1 is limited to mono- or dimethylated lysine, JMJDs can demethylate trimethylated lysine, as shown in Table 2. The histone lysine demethylation by JMJDs modulates the expression of a number of genes. For example, Klose et al. demonstrated that KDM3A (also known as JHDM3A and JMJD2A) demethylates H3K9me3 and H3K36me3 and decreases the expression of the achaete−scute complex homologue 2 gene.[86] On the other hand, Yamane et al. reported that KDM3A (also known as JHDM2A and JMJD1A) specifically demethylates H3K9me1/me2 and facilitates transcriptional activation of androgen receptor target genes.[74]

As shown in Table 2, it has been reported that JMJD lysine demethylases are associated with several disease states, including cancer. For example, Kauffman et al. revealed that KDM4A, a member of the JMJD lysine demethylase family, is involved in bladder cancer initiation and progression.[59] In addition, overexpression of KDM4C, another member of the JMJD lysine demethylase family, increases the expression of Mdm2 oncogene in a manner dependent on KDM4C's demethylase activity, leading to a decrease of p53 tumor suppressor gene product in the cells.[94] Furthermore, the outcome of RNAi-mediated knockdown of KDM4C suggested that this enzyme is associated with cell growth of esophageal squamous cancer,[82] prostate cancer,[35] breast cancer,[92] and primary mediastinal B cell lymphoma and Hodgkin lymphoma.[95] Therefore, selective inhibitors of JMJD lysine demethylases are potential tools for studying the functions of these enzymes and also are candidate therapeutic agents that would be expected to have few side effects.

6. STRUCTURAL STUDIES AND CATALYTIC MECHANISM OF JUMONJI C-CONTAINING LYSINE DEMETHYLASES

Some of the JMJD lysine demethylases, including KDM4A, KDM7A, and KDM7B, are structurally well characterized.[130,132−136] The crystal structures make it clear that α-ketoglutarate coordinates the Fe(II) ion in the active site through its α-carbonyl and carboxylate groups and also forms a hydrogen bond with the other carboxylate group and Tyr or Lys (Figure 6). The structures provided a rationale for the substrate specificity. The X-ray crystal structures of a series of complexes between KDM4A and methylated K9-containing H3 peptides show that the peptide

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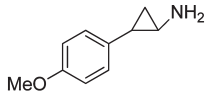
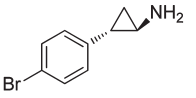


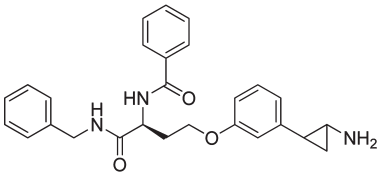
4

K_I (LSD1) = 188 μM
k_{inact}/K_I (LSD1) = 262 $M^{-1}s^{-1}$
K_I (MAO A) = 87 μM
k_{inact}/K_I (MAO A) = 802 $M^{-1}s^{-1}$
K_I (MAO B) = 21 μM
k_{inact}/K_I (MAO B) = 1112 $M^{-1}s^{-1}$



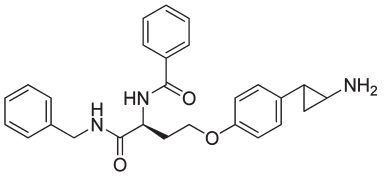
5

K_I (LSD1) = 3.7 μM



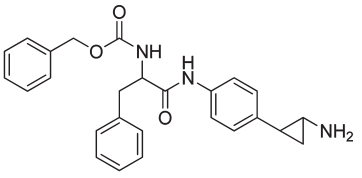
6

K_I (LSD1) = 5.7 μM
k_{inact}/K_I (LSD1) = 2000 $M^{-1}s^{-1}$
K_I (MAO A) = 230 μM
k_{inact}/K_I (MAO A) = 79 $M^{-1}s^{-1}$
K_I (MAO B) = 740 μM
k_{inact}/K_I (MAO B) = 2.6 $M^{-1}s^{-1}$



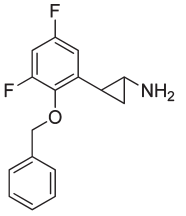
7

K_I (LSD1) = 3.1 μM
k_{inact}/K_I (LSD1) = 670 $M^{-1}s^{-1}$
K_I (MAO A) = 250 μM
k_{inact}/K_I (MAO A) = 70 $M^{-1}s^{-1}$
K_I (MAO B) = 1700 μM
k_{inact}/K_I (MAO B) = 4.1 $M^{-1}s^{-1}$



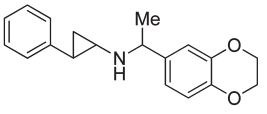
8

K_I (LSD1) = 1.3 μM
K_I (LSD2) = 38 μM
K_I (MAO A) = 1.2 μM
K_I (MAO B) > 133 μM



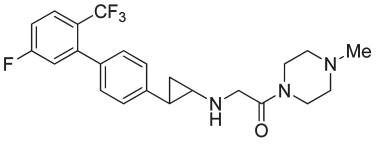
9

K_I (LSD1) = 0.61 μM
k_{inact}/K_I (LSD1) = 4560 $M^{-1}s^{-1}$
K_I (MAO A) = 110 μM
k_{inact}/K_I (MAO A) = 60 $M^{-1}s^{-1}$
K_I (MAO B) = 17 μM
k_{inact}/K_I (MAO B) = 18 $M^{-1}s^{-1}$



10

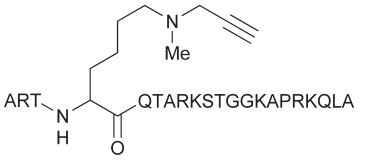
K_I (LSD1) = 5.0 nM
K_I (MAO A) = 16 μM
K_I (MAO B) = 7.4 μM



11

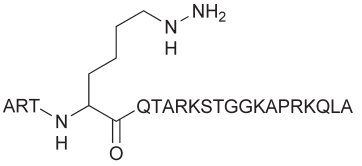
K_I (LSD1) = 9.0 nM
K_I (MAO A) = 15 μM
K_I (MAO B) > 40 μM

Figure 3. PCPA-based LSD1 inhibitors.



12

K_I (LSD1) = 107 nM
k_{inact}/K_I (LSD1) = 1.94 $\mu M^{-1}min^{-1}$



13

K_I (LSD1) = 4.35 nM
k_{inact}/K_I (LSD1) = 56.8 $\mu M^{-1}min^{-1}$

Figure 4. Peptide-based LSD1 inhibitors.

chain lies across a shallow groove, placing the methylated K9 in a relatively large cavity with the methylamino group sufficiently close to the Fe(II) for hydroxylation.[134] The crystal structures also suggested that the methylamino group of the peptide is

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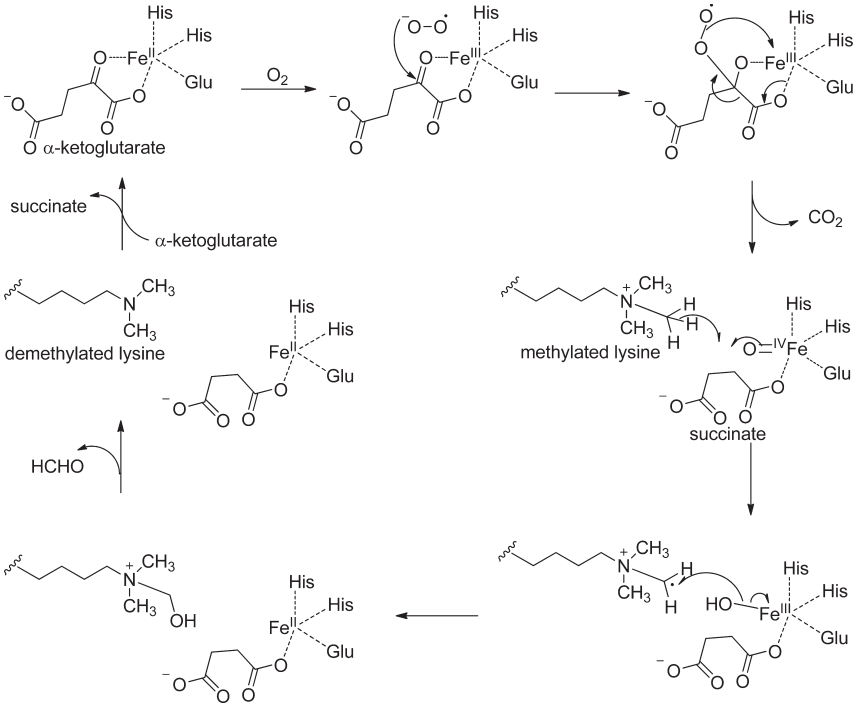
Scheme 3



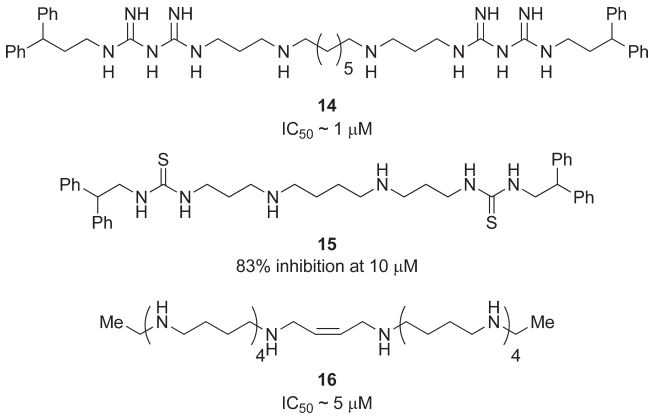


Figure 5. Polyamine-based LSD1 inhibitors.

recognized by oxygen-rich amino acid residues such as Gly, Tyr, Glu, and Ser via CH···O hydrogen bonds between the CH group of the methylamino moiety and the oxygen of the amino acid residues. Lys and Arg, adjacent to the Zn-coordinating residues His and Cys, respectively, are also involved in substrate binding, indicating a role for the Zn-binding site in peptide recognition. In addition, Cheng and co-workers showed that H3K9me2 and H3K27me2 recognition by the Jumonji domain of KDM7B and KDM7A, respectively, requires binding between H3K4me3 and the plant homeodomain of the enzymes.[136]

The catalytic mechanism of histone lysine demethylation by JMJDs has been studied extensively and is well understood[70,82] (Scheme 3). First, single electron transfer from Fe(II) complexed with α-ketoglutarate to molecular oxygen generates Fe(III) and superoxide. Next, the superoxide serves as a nucleophile that attacks the C2 position of α-ketoglutarate, causing decarboxylation of α-ketoglutarate to produce carbon dioxide, succinate, and Fe(IV)=O. This Fe(IV)=O species abstracts hydrogen from the methyl group of the methylated histone lysine residue to produce Fe(III)−OH species. The radical reaction between the methylene radical and Fe(III)−OH generates the hemiaminal intermediate that spontaneously produces formaldehyde and demethylated lysine while regenerating the active Fe(II) center. In contrast to the catalytic mechanism of LSD1, JMJD lysine demethylases do not require the lone pair on the nitrogen of the substrate. Therefore, JMJD lysine demethylases can demethylate mono- and dimethylated lysine, as well as trimethylated lysine.

7. JUMONJI C-CONTAINING LYSINE DEMETHYLASE INHIBITORS

On the basis of the three-dimensional structure and the catalytic mechanism of JMJD histone lysine demethylases mentioned above, a number of JMJD inhibitors have been identified.

As shown in Scheme 3, JMJD lysine demethylases produce not only demethylated lysine but also succinate. Smith et al. showed that high concentrations (>10 mM) of succinate **17** (Figure 7) inhibit JMJD demethylases in a yeast model of paraganglioma.[137] It is likely that succinate **17** inhibits the enzymes through product inhibition by shifting the equilibrium in the reaction, enzyme-α-ketoglutarate + methylated lysine ↔ enzyme-succinate + demethylated lysine, toward the reactants.

Recently, it has been reported that the Ni(II) ion causes inhibition of KDM3A by replacing Fe(II) in the active site of the enzyme (IC_{50} = 25 μM).[138] In human epithelial BEAS-2B cells, Ni(II) exposure increased the level of H3K9me2 at the promoter of *Spy2*, a KDM3A-targeted gene, by inhibiting KDM3A.

Analogues of α-ketoglutarate have been reported to inhibit JMJD proteins. These analogues are thought to be competitive inhibitors with respect to α-ketoglutarate. The oncometabolite

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Table 2. JMJD Lysine Demethylase Family

name	alternative name	substrate	transcription	link to diseases	refs
KDM2A	JHDM1A, FBXL11	H3K36me 1/2	repression	not reported	70
KDM2B	JHDM1B, FBXL10	H3K4me3,H3 K36me 1/2	repression	required for initiation and maintenance of acute myeloid leukemia	70−73
KDM3A	JMJD1A, JHDM2A, TSGA	H3K9me 1/2	activation	regulates metabolic gene expression and obesity resistance; enhances tumor cell growth	74−78
KDM3B	JMJD1B, JHDM2B, 5qNCA	H3K9me 1/2	activation	suppresses MUTZ-1 cell growth	13, 79
KDM3C	JMJD1C, JHDM2C, TRIP8	H3K9me 1/2	activation	expressed in diffuse-type gastric cancer	80, 81
KDM4A	JMJD2A, JHDM3A	H1.4K26me3	unknown	overexpressed in prostate cancer; involved in bladder cancer initiation	59, 82−88
		H3K9me 2/3	activation	and progression; regulates Kaposi's sarcoma-associated herpesvirus	
		H3K36me 2/3	repression	replication; promotes cardic hypertrophy	
KDM4B	JMJD2B, JHDM3B	H1.4K26me3	unknown	overexpressed in prostate cancer, breast cancer and desmoplastic	82−85, 89−91
		H3K9me 2/3	activation	medulloblastoma; enhances breast cancer cell growth	
		H3K36me 2/3	repression		
KDM4C	JMJD2C, JHDM3C, GASC1	H1.4K26me3	unknown	overexpressed/amplified in prostate cancer, esophageal squamous	35, 82−85, 90, 92−95
		H3K9me 2/3	activation	cell carcinoma, desmoplastic medulloblastoma, metastatic	
		H3K36me 2/3	repression	lung sarcomatoid carcinoma, breast cancer, primary mediastinal B cell lymphoma, and Hodgkin lymphoma; increases expression of Mdm2 oncogene	
KDM4D	JMJD2D, KIAA0780	H1.4K26me 2/3	unknown	not reported	84, 85
		H3K9me 1/2/3	unknown		
		H3K36me 2/3	repression		
KDM5A	JARID1A, RBP2	H3K4me 2/3	repression	induces acute myeloid leukemia; overexpressed in gastric cancer and prevents senescence of malignant cells; involved in drug-tolerant state in cancer	96−100
KDM5B	JARID1B, PLU-1	H3K4me 2/3	repression	overexpressed in breast, testis, and prostate cancer; involved in cancer cell growth	101−105
KDM5C	JARID1C, SMCX	H3K4me 2/3	repression	mutated in X-linked mental retardation; involved in neuronal survival and dendritic development; mutated in renal carcinoma	106−108
KDM5D	JARID1D, SMCY	H3K4me 2/3	repression	not reported	109
KDM6A	UTX	H3K27me 2/3	activation	mutated in cancer	110−113
KDM6B	JMJD3	H3K27me 2/3	activation	involved in inflammatory signaling cascades; acts as a tumor suppressor; overexpressed in Hodgkin's lymphoma	110, 111, 114−116
KDM7A	JHDM1D, KIAA1718	H3K9me2, H3K27me2	activation	not reported	117, 118
KDM7B	JHDM1F, PHF8, KIAA1111	H3K9me 1/2, H3K27me2, H4K20me1	activation	mutated in X-linked mental retardation	118−124
		H3K36me2	repression		
KDM8	JMJD5	H3K36me2	activation	overexpressed in cancer; enhances cancer cell growth	125
NO66	MAPJD	H3K4me 1/2/3, H3K36me 2/3	repression	overexpressed in non-small-cell lung cancer; enhances cancer cell growth	126, 127
Mina53	MDIG, NO52	H3K9me3	activation	overexpressed in lymphoma, renal cell carcinoma, neuroblastoma, gastric carcinoma, lung cancer, and hepatocellular carcinoma	128, 129
PHF2	GRC5, JHDM1E, KIAA0062, MGC176680	H3K9me 1/2	activation	not reported	130, 131

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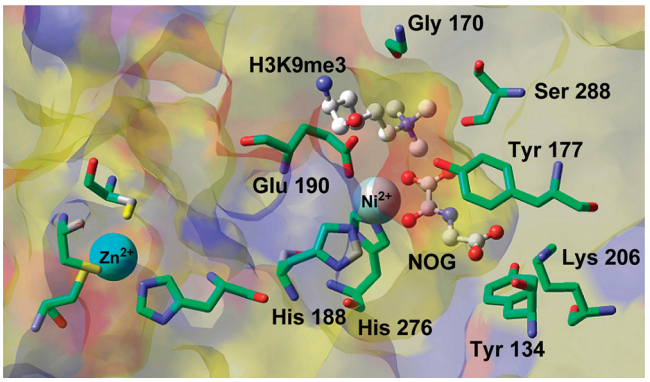


Figure 6. X-ray crystal structure of JMJD2A complexed with *N*-oxalylglycine (NOG), the amide analogue of α-ketoglutarate, and an H3K9me3 peptide (PDB code 2OQ6). Amino acid residues of JMJD2A are displayed as tube models, and the histone H3 peptide and NOG are displayed as ball-and-stick models. Fe(II) is replaced by Ni(II).

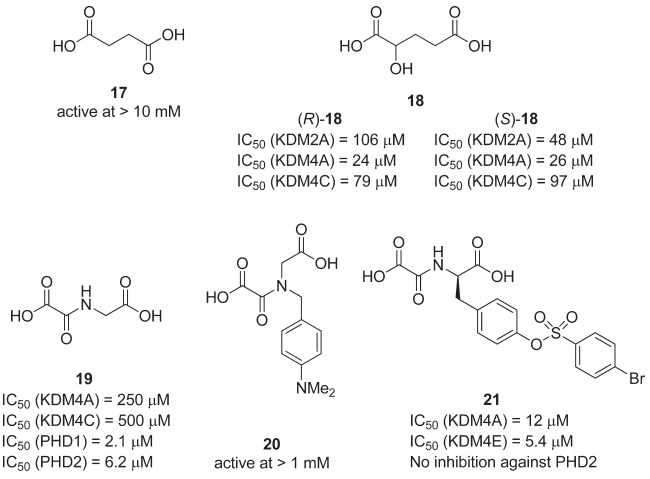


Figure 7. Structures of succinate **17**, α-hydroxyglutarate **18**, NOG **19**, and NOG's derivatives **20** and **21**.

α-hydroxyglutarate **18** (Figure 7), a reduced analogue of α-ketoglutarate, inhibited KDM2A, KDM4A, and KDM4C with IC_{50} ranging from 24 to 106 μM.[139] *N*-Oxalylglycine (NOG, **19**) (Figure 7), an amide analogue of α-ketoglutarate, has been reported to inhibit JMJD proteins in vitro.[82] Although NOG (**19**) itself does not display activity in cells because of its poor membrane permeability, owing to its high polarity, 2.5 mM dimethyloxalylglycine (DMOG), the dimethyl ester prodrug of NOG, enhanced the methylation levels of H3K9 and H3K36, indicating that **19** generated intracellularly from DMOG inside the cells represses the demethylation activity of JMJDs.[140] The X-ray crystal structure of KDM4A complexed with **19** was reported.[133] It showed that the oxalyl group of **19** coordinates in a bidentate manner to Fe(II), and the other carboxyl group forms a hydrogen bond with Tyr132 in the active center of KDM4A. On the basis of the crystal structure, **19** derivatives such as **20** and **21** (Figure 7) have been designed in attempts to find JMJD lysine demethylase inhibitors more potent than **19**.[140−142] In particular, although α-hydroxyglutarate **18** and **19** inhibit other Fe(II)/α-ketoglutarate-dependent oxygenases, such as prolyl hydroxylase domain-containing proteins (PHDs) and factor-inhibiting hypoxia-inducible factor (FIH), *N*-oxalyl-D-tyrosine derivative

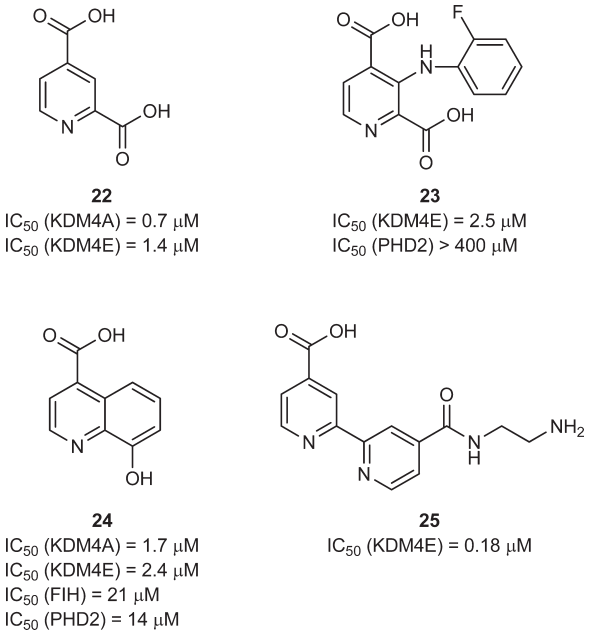
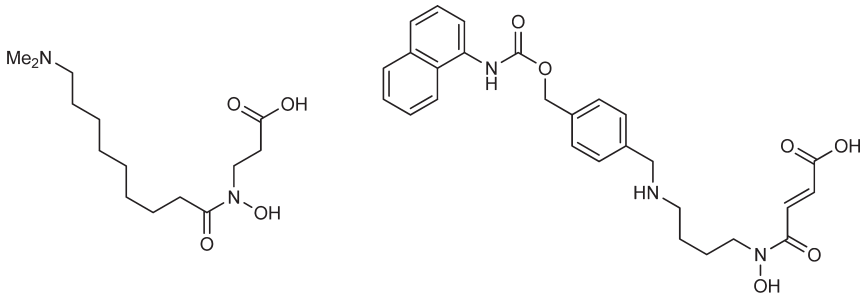


Figure 8. Pyridine-based JMJD histone demethylase inhibitors.

21 showed selective inhibition of KDM4 over PHD2, another Fe(II)/α-ketoglutarate-dependent enzyme that hydroxylates hypoxia-inducible factor.[141] The crystal structure of the KDM4A complex with an analogue of compound **21** showed that the tyrosinyl side chain of the inhibitor interacts with hydrophobic amino acid residues (Ile71, Tyr132, Tyr177, Phe185, and the alkyl chain of Lys241) which form a subpocket at the KDM4A active site.[141]

2,4-Pyridinedicarboxylic acid (PCA, **22**) (Figure 8), which inhibits other Fe(II)/α-ketoglutarate-dependent oxygenases, was also reported to be a potent inhibitor of KDM4A and KDM4E (IC_{50} = 0.7−4.7 μM).[142] The X-ray crystal structure of **22** bound to KDM4A revealed that **22** inhibits KDM4 in a binding mode similar to that of **19**. It binds to Ni(II) [which replaces Fe(II)] in a bidentate manner through its nitrogen atom and 2-carboxylate oxygen. The 4-carboxylate oxygen of **22** forms hydrogen bonds with NH of Lys241 and OH of Tyr177. The pyridine ring of **22** forms hydrophobic interactions with Tyr177, Phe185, and Trp208. The structural analysis suggested that a substituent at the C-3 position of **22** can interact with amino acid residues of KDM4A, such as Tyr177, while the active site cavity of PHD2 is not capacious enough to accommodate C-3-substituted PCA. Schofield and co-workers identified C-3-substituted PCAs such as **23** (Figure 8) by means of structure-based drug design.[143] Compound **23** was an inhibitor with selectivity for KDM4E over PHD2 (KDM4E IC_{50} = 2.5 μM; PHD2 IC_{50} > 400 μM), demonstrating that structure-based drug design can lead to the identification of inhibitors with selectivity for JMJD over other Fe(II)/α-ketoglutarate-dependent oxygenases.

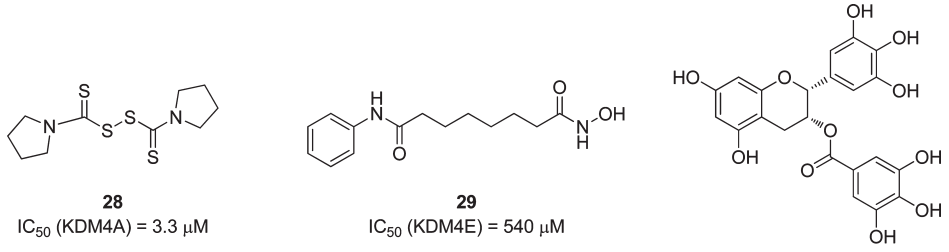
The PCA-related compound SID 85736331 (**24**) (Figure 8) was discovered as a KDM4 inhibitor (KDM4A IC_{50} = 1.7 μM; KDM4E IC_{50} = 2.4 μM) by means of high-throughput screening of about 236 000 compounds.[144] The structure of KDM4A complexed with compound **24** revealed that **24** is positioned in a similar location to **19** and **22**; it coordinates with Ni(II) in the active site in a bidentate fashion via its quinoline nitrogen and 8-hydroxyl group. The C-5-carboxylate group of compound **24** is



26
IC_{50} (KDM4A) = 3.0 μM
IC_{50} (KDM4C) = 1.0 μM
IC_{50} (PHD1) > 100 μM
IC_{50} (PHD2) > 100 μM

27
IC_{50} (KDM4A) = 4.3 μM
IC_{50} (KDM4C) = 3.4 μM
IC_{50} (KDM4E) = 5.9 μM
IC_{50} (KDM6B) = 43 μM
IC_{50} (KDM7B) = 10 μM
IC_{50} (PHD1) = 54 μM
IC_{50} (PHD2) = 83 μM
IC_{50} (PHD3) = 31 μM
IC_{50} (FIH) = 22 μM

Figure 9. Hydroxamate-based JMJD histone demethylase inhibitors.



28
IC_{50} (KDM4A) = 3.3 μM

29
IC_{50} (KDM4E) = 540 μM

30
IC_{50} (KDM4E) = 3.0 μM

Figure 10. Structures of disulfiram analogue **28**, vorinostat **29**, and epigallocatechin gallate **30**.

positioned to interact with Lys206 and Tyr132. Compound **24** also showed dose-dependent KDM4A-inhibitory activity in cells at concentrations ranging from 100 to 300 μM and restored the trimethylation pattern at the H3K9 locus in cells overexpressing KDM4A demethylase. Its activity was 40-fold stronger than that of DMOG.

Pyridine compounds of another type, such as bipyridine **25** (Figure 8), have recently been reported as KDM4 inhibitors.[145] Compound **25** inhibited KDM4E with an IC_{50} of 0.18 μM. The crystal structure of the KDM4A complex with **25** revealed that compound **25** binds to the active site metal by bidentate chelation through its two pyridine nitrogens. The carboxylate of **25** is positioned to interact with Lys206 and Tyr132 in a manner analogous to **19**, **22**, and **24**. The amide nitrogen of **25** is positioned to form two hydrogen bonds with the phenolic oxygen atom of Tyr177 and the backbone carbonyl oxygen atom of Glu169 via water molecules. Electrostatic interaction of the cationic amino group of **25** with Asp135 was also observed.

NCDM-32 (**26**) (Figure 9) was designed based on the crystal structure of KDM4A and a homology model of KDM4C complexed with **19** and a histone trimethylated lysine peptide.[146] Compound **26** inhibited KDM4A and KDM4C with IC_{50} of 3.0 and 1.0 μM, respectively, showing 500-fold greater KDM4C-inhibitory activity and more than 9100-fold greater KDM4C/PHD selectivity compared with the lead compound **19**. Furthermore, the ester prodrug of **26** showed synergistic growth inhibition of cancer cells in combination with **7**, an inhibitor of LSD1. Wang and co-workers have recently reported a novel JMJD-selective inhibitor **27** (Figure 9) which was also identified by means of structure-based drug design.[147] In enzyme assays, compound **27** inhibited the subfamily of trimethyllysine demethylases, such as KDM4A and KDM4C, preferentially over other Fe(II)/α-ketoglutarate-dependent oxygenases such as PHD1 and PHD2. More importantly, methylstat, the methyl ester prodrug of **27**, selectively inhibited JMJD demethylases in cells and showed growth inhibition of esophageal carcinoma KYSE150 cells, in which KDM4C is highly expressed. The cancer cell growth-inhibitory activity of the prodrug of **26** and methylstat suggested that JMJD demethylase inhibitors have clinical potential for anticancer chemotherapy.

Schofield and co-workers demonstrated that disulfiram analogues such as compound **28** (Figure 10) inhibit JMJD2A by removing Zn ion from the Zn-binding site of JMJD2A.[148] Their work suggested that Zn removal has potential for achieving selective inhibition of the JMJD2 demethylases over those family members that do not have a Zn-binding site.

Hydroxamic acids such as vorinostat **29** (Figure 9) and catechols such as epigallocatechin gallate **30** (Figure 10) have been reported to inhibit JMJD2 demethylases, although they also inhibit other enzymes, including HDACs and DNA methyltransferases.[142,149,150]

8. CONCLUSION

Since the discovery of LSD1 in 2004, a number of lysine demethylases have been identified. There is now strong evidence that at least some of the lysine demethylases, including LSD1 and KDM4C, are associated with disease states including cancer, herpes simplex, and metabolic diseases. Thus, lysine demethylases

have emerged as attractive targets in drug development. Efforts by many groups to find lysine demethylase inhibitors have led to the identification of several classes of inhibitors, as described here. However, the activities (IC_{50} or K_I of most reported lysine demethylase inhibitors hover at a level of micromolar. It is hoped that more potent and selective lysine demethylase inhibitors will be identified by structure based drug design as is the case with other protein ligands. To date, the X-ray crystal structures of LSD1, KDM4A, KDM4C, KDM7A, and KDM7B have been published. These crystal structures should pave the way for the design of more potent, isozyme-selective inhibitors, which should be useful not only as tools for detailed elucidation of the biological functions of the isozymes but also in the development of therapeutic agents with few side effects.

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ABBREVIATIONS USED

HKMT, histone lysine methyltransferase; KDM, histone lysine demethylase; LSD, lysine-specific demethylase; JMJD, Jumonji domain-containing protein; FAD, flavin adenine dinucleotide; CoREST, the RE1-silencing transcription factor corepressor; HDAC, histone deacetylase; SYN, synapsin; MuAchR4, muscarinic acetylcholine receptor 4; HSV, herpes simplex viruses; VZV, varicella zoster viruses; HCF-1, host cell factor 1; MAO, monoamine oxidase; PCPA, 2-phenylcyclopropylamine; SFRP, secreted frizzle-related protein; NOG, *N*-oxalylglycine; DMOG, dimethyloxalylglycine; PHD, prolyl hydroxylase domain-containing protein; FIH, factor-inhibiting hypoxia-inducible factor; PCA, 2,4-pyridinedicarboxylic acid

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