Review

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Advances toward LSD1 inhibitors for cancer therapy

LSD1 has become an important biologically validated epigenetic target for cancer therapy since its identification in 2004. LSD1 mediates many cellular signaling pathways and is involved in the initiation and development of cancers. Aberrant overexpression of LSD1 has been observed in different types of cancers, and inactivation by small molecules suppresses cancer cell differentiation, proliferation, invasion and migration. To date, a large number of LSD1 inhibitors have been reported, RG6016, GSK-2879552, INCB059872, IMG-7289 and CC-90011 are currently undergoing clinical assessment for the treatment of acute myeloid leukemia, small lung cancer cell, etc. In this review, we briefly highlight recent advances of LSD1 inhibitors mainly covering the literatures from 2015 to 2017 and tentatively propose our perspectives on the design of new LSD1 inhibitors for cancer therapy.

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Biological roles of LSD1

Historically, the histone methylation has long been recognized as an irreversible process prior to the identification of lysine specific histone demethylase 1 (LSD1, also known as KDM1A) by Professor Yang Shi in 2004 [1]. As the first histone demethylase, LSD1 catalyzes the demethylation of mono- and di-methylated K4 or K9 on histone H3 (H3K4me1/2 & H3K9me1/2) under diverse biological settings using the FAD as a cofactor [2,3,4]. Additionally, LSD1 can also demethylate many other nonhistone substrates such as p53, DNMT1, STAT3, E2F1, etc. [5,6,7]. Mounting evidences have shown that LSD1 mediates many cellular signaling pathways [8,9,10] and plays critical roles in regulating fundamental cellular processes (for part of LSD1-mediated biological processes, see Figure 1A) [11,12,13]. The diverse biological roles of LSD1 may explain why its dysfunction is associated with initiation and development of several diseases such as cancers, neurodegenerative diseases, cardiovascular diseases, inflammation, viral infections, etc. (Figure 1B) [14]. Aberrant overexpression of LSD1 has been observed in various human cancer cells (Figure 1C) and is closely associated with differentiation, proliferation, migration, invasion and poor prognosis [15,16,17,18]. Inactivation by small molecules or RNAi-mediated downregulation of LSD1 inhibited cancer cell differentiation, proliferation, invasion and migration, and tumor growth in different types of animal models [19,20,21,22,23,24]. These findings underscore the biological importance of LSD1 and therapeutic potential of LSD1 inhibitors for cancer therapy.

Structural basis for designing new LSD1 inhibitors

LSD1 consists of 852 amino acids, which form the N-terminal small α-helical domain (SWIRM), the amine oxidase like (AOL) catalytic domain containing noncovalent

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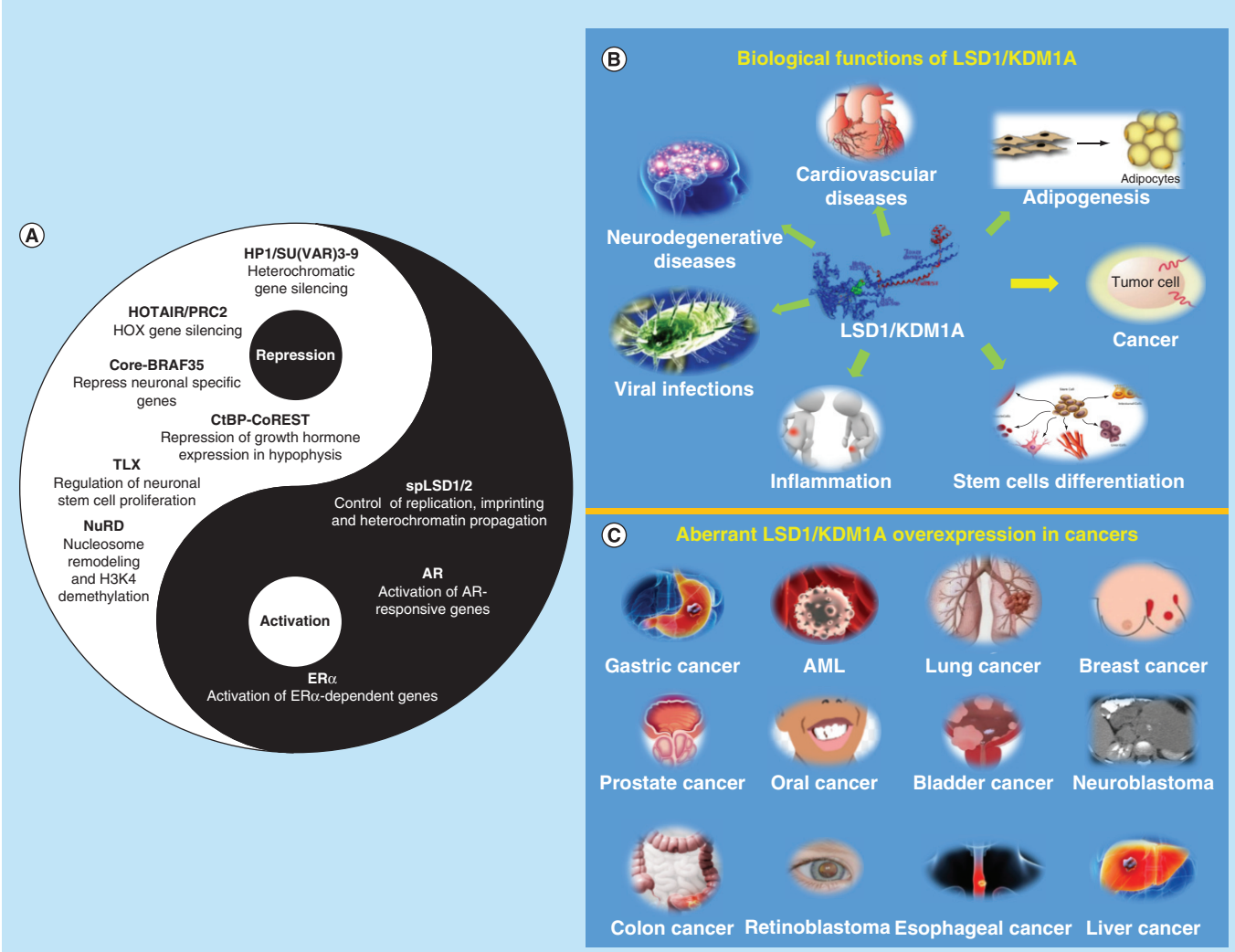

Figure 1. Functional roles of LSD1 in normal physiological processes and cancers. (A) LSD1-mediated biological processes; **(B)** Types of disease where LSD1 is involved; **(C)** Cancer types where LSD1 is aberrantly overexpressed.

flavin adenine dinucleotide (FAD)-binding site as well as substrate-binding site, and the TOWER domain (Figure 2A) [25,26,27]. To date, over 30 crystal structures of LSD1 have been deposited in RCSB Protein Data Bank with the highest resolution of 2.8 Å (PDB code: 5L3E) [28]. The SWIRM domain in the *N*-terminus of LSD1 contains six α-helices and is also involved in the interaction with an N-terminal tail of histone H3. The AOL domain in the C-terminus of LSD1 is a highly conserved functional region and shares 20% sequence similarity with that of the FAD-dependent monoamine oxidases (MAOs) and polyamine oxidases. The FAD is buried into the deepest hydrophobic part of the pocket and interacts with Lys661 [29]. The Lys661 deprotonates the methylated histone lysine through a water bridge, thus allowing hydride being transferred onto the FAD for oxidative demethylation. Mutations at Lys661 abolish the demethylase activity of LSD1. The FAD-binding site is highly akin to that of other MAOs, while the substrate-binding region is larger and relatively hydrophilic in contrast to that of MAOs. Therefore, this structural difference in the substrate-binding site provides a basis for designing selective inhibitors toward LSD1 over MAOs. Also, the large size of H3-binding site requires that the histone tail must be directed to appreciate position for demethylation, thus making the design of potent reversible LSD1 inhibitors challenging (Figure 2B). The rim of AOL domain is lined with negatively charged residues (e.g., Asp555, Asp556), which provide electrostatic interactions with substrates. The TOWER domain features a long helix-turn-helix structure and comprises binding surfaces for LSD1 partners (e.g., CoREST, HDAC1/2,

snail, AR, etc.) [30,31,32,33,34], which exert great impact on the demethylase activity. Defined features of the histone peptide-binding site in LSD1 are shown in Figure 2B, highlighting the presence of subpockets (shown in bold) in the substrate-binding region of LSD1 that interact with Thr6, Arg8, Lys9 and Thr11 residues of H3 side chain and also with the NH_2 group of Ala1 (N-term pocket) [29]. Moreover, the intrapeptide hydrogen bonds (shown as dashed lines) between the side chain of Arg2 and other residues of H3 peptide are crucial for stabilizing the conformation of the LSD1-bound H3 peptide. These interactions direct the methylated Lys4 to the FAD for oxidative demethylation and are responsible for the LSD1 specificity for H3K4. The design of small molecules (e.g., mimicking the Arg2 residue) interrupting the stabilized network could be a viable strategy for designing LSD1 inhibitors.

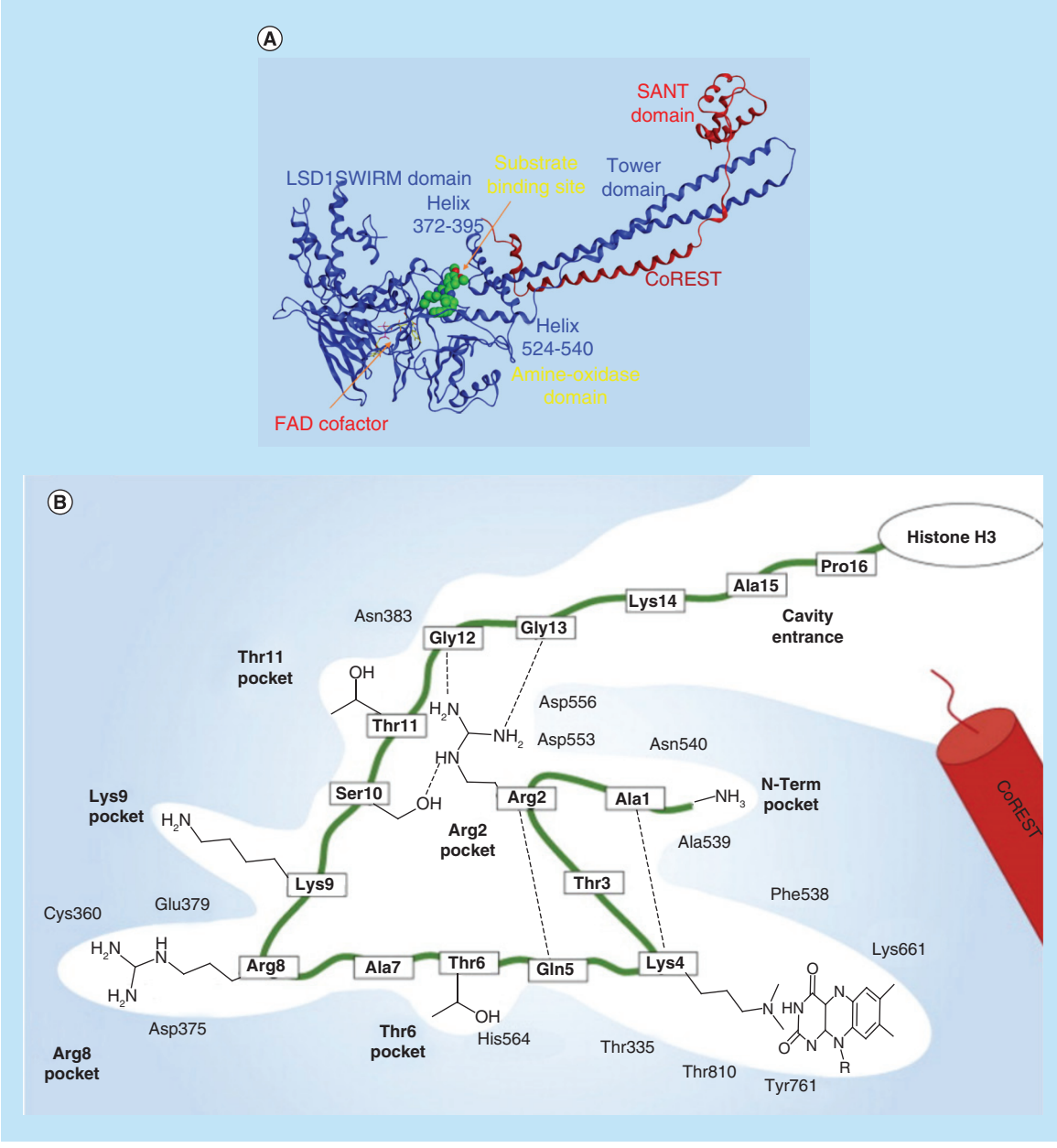

Figure 2. Co-crystal structure of LSD1-CoREST-E11 complex and peptide-binding regions. (A) Crystal structure of LSD1-CoREST in complex with reversible inhibitor **E11** (PDB code: 5L3E); **(B)** Defining features of the histone peptide-binding site in LSD1. The peptide-binding pocket is near to the LSD1–coREST (red) interface.
CoREST: RE1-Silencing transcriptional corepressor 1.
(B) Adapted with permission from reference [29] © Elsevier Ltd (2008).

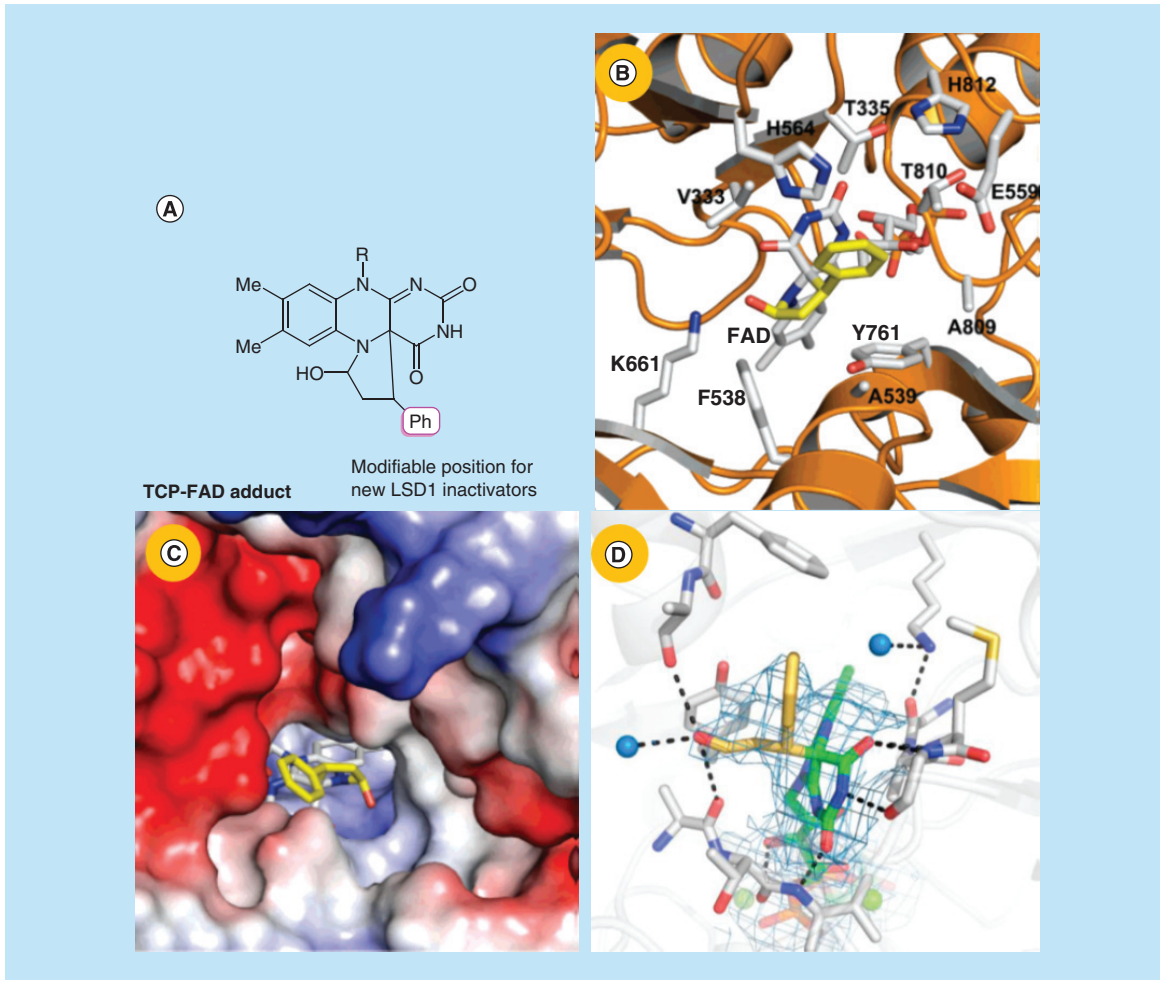

Figure 3. Binding poses of the tranylcypromine-FAD adducts in the active site of lysine specific histone demethylase 1. (A) The structure of TCP-FAD adduct. **(B)** Binding pose of the TCP-FAD adduct in the active site of LSD1. **(C)** The surface maps of the TCP-FAD adduct, the negatively charged regions are highlighted in red, and the hydrophobic regions are shown in blue. **(D)** The cocrystal structure of GSK2699537-FAD adduct bound to LSD1-CoREST protein.
FAD: Flavin adenine dinucleotide; LSD1: Histone lysine specific demethylase 1; TCP: Tranylcypromine.
Figure **3B** and **C** was adapted with permission from [22] © Future Medicine Ltd (2016).
Figure **3D** was adapted with permission from [39] © Elsevier Inc (2015).

Recent advances of LSD1 inhibitors

Irreversible LSD1 inhibitors

Small-molecule inhibitors of MAOs including tranylcypromine, pargyline and phenelzine were initially found to be able to inactivate LSD1 weakly [20]. The tranylcypromine (abbreviated as TCP or 2-PCPA), as a MAO inhibitor used in clinic for the treatment of depression, was identified as an irreversible and nonselective mechanism-based inactivator of LSD1 through forming covalent TCP-FAD adducts as shown in Figure 3A [35,36,37]. The adduct is nested in a hydrophobic cavity formed by H564, T335, T810, V333, A809 and Y761 residues, while the phenyl ring forms weak van der Waals contacts with T335 and T810 residues and has no interaction with surrounding hydrophobic residues (Figure 3B). As shown in Figure 3C, the phenyl ring of the adduct is directed to the large substrate-binding region surrounded by negatively charged residues, suggesting that the introduction of relatively large hydrophobic substituents, especially those bearing additional basic moiety [38], to the phenyl ring may improve potency through forming additional electrostatic interactions. To clearly show the binding models of TCP-based LSD1 inhibitors in the active site, the cocrystal structure of GSK2699537-FAD adduct bound to LSD1-CoREST protein is depicted in Figure 3D, showing key hydrogen interactions with nearby Val333, Met332, Val811, Ala539 and Ala809 residues [39].

Based on the structural features of LSD1 cocrystal structures, industrial companies and academic groups

have been devoted to identifying potent TCP-based LSD1 inhibitors for cancer therapy. To date, a large number of irreversible TCP-based LSD1 inhibitors have been discovered [20,22]. Among these inhibitors, three irreversible LSD1 inhibitors RG6016 (also known as ORY-1001 and RO7051790) [40], GSK-2879552 [39,41], IMG-7289, CC-90011 and INCB059872 [42,43] alone or in combination with other therapeutic agents, are currently undergoing advanced preclinical/clinical assessment for cancer therapy, such as acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), acute lymphoblastic leukemia (ALL) and small-cell lung cancer (SCLC) (Table 1). The success of RG6016 and GSK-2879552 makes the TCP a privileged scaffold for designing potent LSD1 inhibitors. For detailed advances on TCP-based irreversible inhibitors for cancer therapy, please refer to our recent reviews [20,22].

Reversible LSD1 inhibitors

Natural LSD1 inhibitors

Natural products (NPs) have long been recognized as rich sources for identifying new therapeutic agents. Some NPs have been reported to be able to inhibit LSD1 (Figure 4). Based on the structural similarities between LSD1 and MAOs, Willman *et al.* identified the first natural LSD1 inhibitor Namoline (reversible MAO-A/B inhibitor previously identified from a focused, NP-inspired library) after screening a library of natural γ-pyrone compound library (around 705 compounds) using a horseradish peroxidase (HRP)-coupled assay [44]. Namoline inhibited LSD1 reversibly (IC_{50} = 51 μM), robustly demethylated H3K4me1/2, impaired the androgen receptor (AR)-induced proliferation and tumor growth bearing androgen-sensitive human prostate adenocarcinoma cells (LNCaP cells). However, it should be noted that namoline caused certain side effects such as the weight loss and liver toxicity, and namoline analogs did not show clear structure–activity relationships (SARs) possibly due to its low potency range. In 2013, Yang *et al.* investigated the effects of natural polyphenols on the LSD1 activity [45], and found that Resveratrol inhibited LSD1 with an IC_{50} value of 15 μM and was more potent than TCP, while curcumin, luteolin, myricetin and quercetin showed weak inhibition toward LSD1, the apigenin, genistein and epigallocatechin gallate (EGCG; structures not shown here) were even found to be devoid of the activity. Recently, our group found that baicalin reversibly inactivated LSD1 (IC_{50} =

Table 1. Lysine specific histone demethylase 1 inhibitors in clinical trials for cancer therapy.

Drugs	Sponsor	Phase	Trial number	Diseases
RG6016	Oryzon/Roche	Phase I/II	NA	AML
		Phase I	NCT02913443	SCLC
		Preclinical	NA	ALL, solid tumors
TCP/ATRA	University of Miami	Phase I	NCT02273102	AML; MDS
	Martin-Luther-Universität Halle-Wittenberg	Phase I/II	NCT02261779	Relapsed/refractory AML
TCP/ATRA/cytarabine	Ulrike Kohlweyer	Phase I/II	NCT02717884	Non-M3 AML
GSK-2879552	GlaxoSmithKline	Phase I	NCT02034123	Relapsed/refractory SCLC
			NCT02177812	AML
GSK-2879552/Azacitidine		Phase I/II	NCT02929498	High-risk MDS
INCB059872	Incyte Corporation	Phase I/II	NCT02712905	Advanced malignancies
IMG-7289/ATRA	Imago BioSciences	Phase I	NCT02842827	AML; MDS
CC-90011	Celgene Corporation	Phase I	NCT02875223	Relapsed/refractory solid tumors and non-Hodgkin's lymphomas

NA: Data are not available on the ClinicalTrials.gov website, and the related data are excerpted from the Oryzon website [70].
ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; ATRA: All *trans* retinoic acid; MDS: Myelodysplastic syndromes; SCLC: Small-cell-lung cancer; TCP: Tranylcypromine.

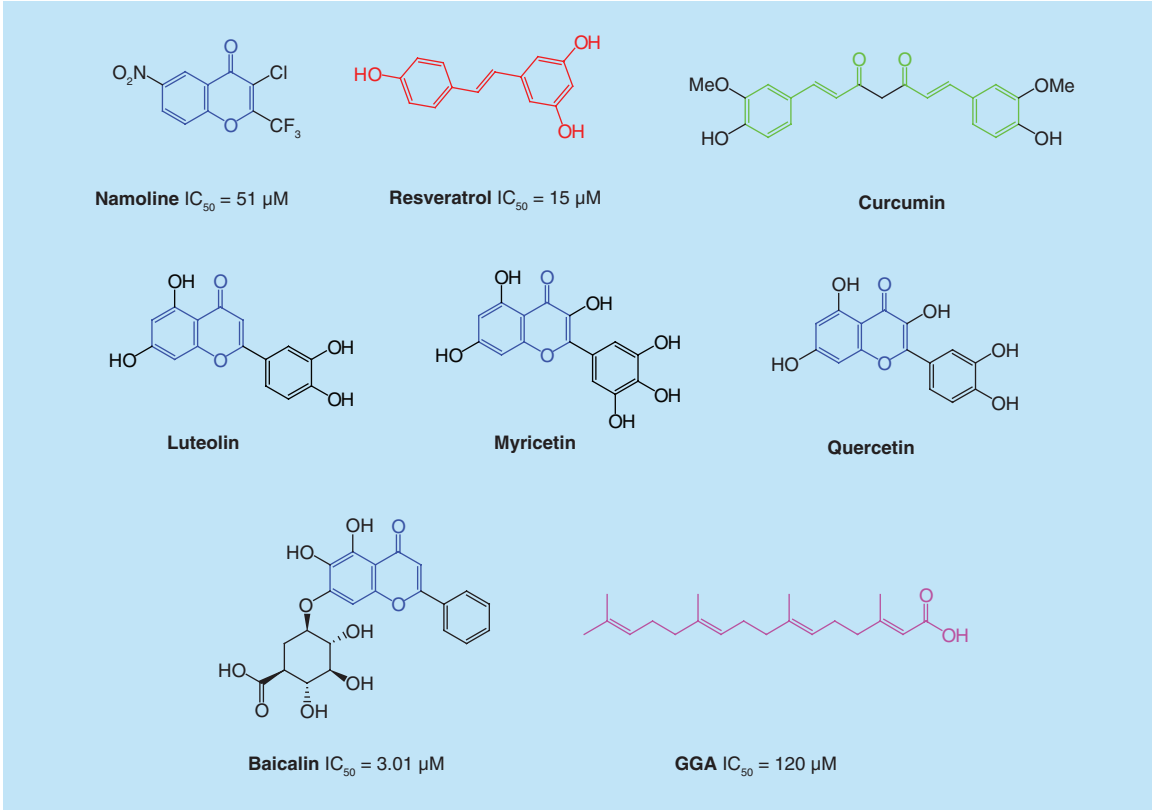

Figure 4. Natural lysine specific histone demethylase 1 inhibitors. The core structures are highlighted in different colors.

3.01 μM) and inhibited growth of LSD1 overexpressed MGC-803 cells moderately (IC_{50} = 8.78 μM) [46], baicalein without the sugar moiety was inactive against LSD1, highlighting the importance of the sugar group for the activity. Further mechanistic studies showed that baicalin downregulated expression of H3K4me2, significantly increased amount of cellular biomaker CD86 mRNA, and inhibited migration of MGC-803 cells accompanied with expression changes of E-Cadherin and N-Cadherin. Shidoji *et al.* reported that the natural acyclic diterpenoid geranylgeranoic acid showed similar LSD1 inhibitory effect with TCP (IC_{50} = 120 μM), induced expression of NTRK2 gene and upregulated H3K4me2 in the regulatory regions of the NTRK2 gene in SH-SY5Y cells [47].

Apart from above natural LSD1 inhibitors, Speranzini recently identified two natural cyclic peptides Polymyxins B and E featuring a linear head and five positively charged propanamine units (highlighted in blue in Figure 5), which competitively inactivated LSD1-CoREST with the K_i values of 157 and 193 nM, respectively [28]. However, polymyxin E did not show remarkable effects on either the cell growth of MV4-11 cells or H3K4/H3K9 methylation, possibly because of its poor permeability across plasma membrane, thus limiting the cellular efficacy. The cocrystal structures of polymyxins E and B/LSD1 revealed that polymyxins E and B formed electrostatic interactions with nearby negatively charged residues (highlighted in red in Figure 5B) at the entrance of the H3 tail-binding cleft, and were relatively distant from the FAD.

Non-natural LSD1 inhibitors

Through the virtual screening of the Maybridge Hitfinder 5 compound library, the Woster group identified amidoximes that inhibited LSD1 moderately, induced H3K4 demethylation in Calu-6 lung carcinoma cells and increased cellular levels of SFRP 2, H-cadherin and the transcription factor GATA4 [48]. Among these compounds, compound **1** (Figure 6) inactivated LSD1 with an IC_{50} value of 16.8 μM. Inspired by the inhibitory effects of resveratrol and amidoxime small-molecule fragments on LSD1, our group recently designed a new library of resveratrol analogs through the molecular hybridization strategy, which were proved to be highly potent and reversible LSD1 inhibitors [49]. Among these compounds, compound **2** was the most potent one with an IC_{50} value of 121 nM. Compound **2** dose-dependently increased accumulation of H3K4me2 and CD86 mRNA in MGC-803 cells, confirming the cellular

LSD1 inhibitory effect of the resveratrol derivatives. By utilizing the same strategy, our group designed a collection of 1,2,3-triazole-dithiocarbamate conjugates by combining the biologically important triazole and dithiocarbamate fragments, which displayed reversible and FAD competitive LSD1 inhibition [50]. The most potent compound **3** inhibited LSD1 with an IC_{50} value of 2.1 μM (K_d = 0.35 μM) and showed high selectivity to LSD1 over MAOs (MAOs IC_{50} >1250 μM, selectivity index >600). Compound **3** induced apoptosis, inhibited growth, migration and evasion of MGC-803 cells overexpressing LSD1 and exhibited robust *in vivo* antitumor efficacy against MGC-803 xenograft models without severe toxicity. Based on the 1,2,3-triazole-dithiocarbamate scaffold, we then introduced the bioactive coumarin moiety (previously identified as highly potent fragments toward MAOs) in place of the phenyl ring in compound **3**, generating a series of new hybrids, which

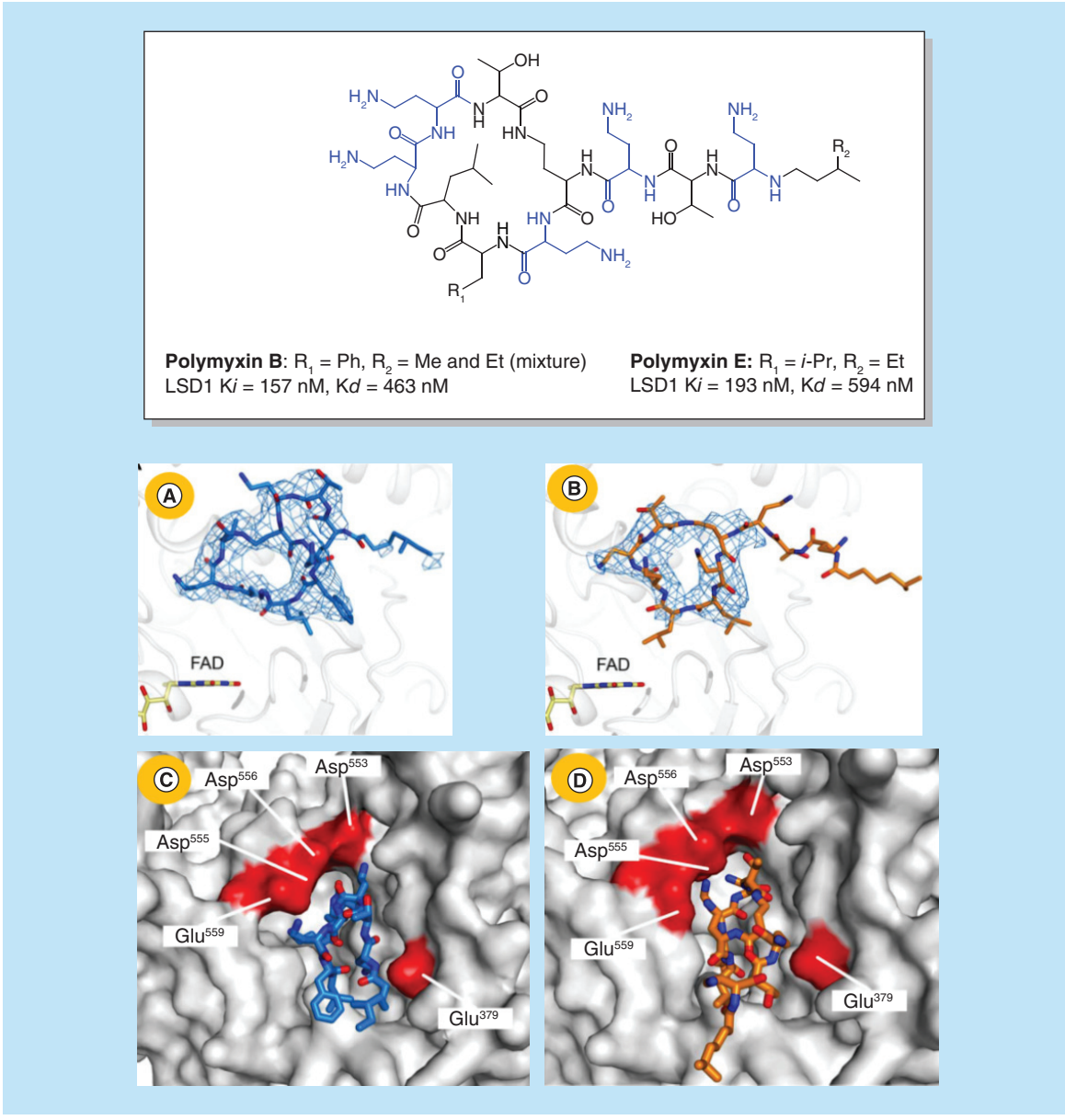

Figure 5. Chemical structures of Polymyxins B and E and their binding models in the active site of LSD1.
(A) Natural polymyxins B and E shown in blue and orange, respectively show similar binding conformation in the active site of LSD1. **(B)** Polymyxins B and E form electrostatic interactions with surrounding negatively charged residues (highlighted in red on the protein surface). **(C & D)** LSD1-bound polymyxins form electrostatic interactions with surrounding negatively charged residues (highlighted in red).
LSD1: Lysine specific histone demethylase 1.
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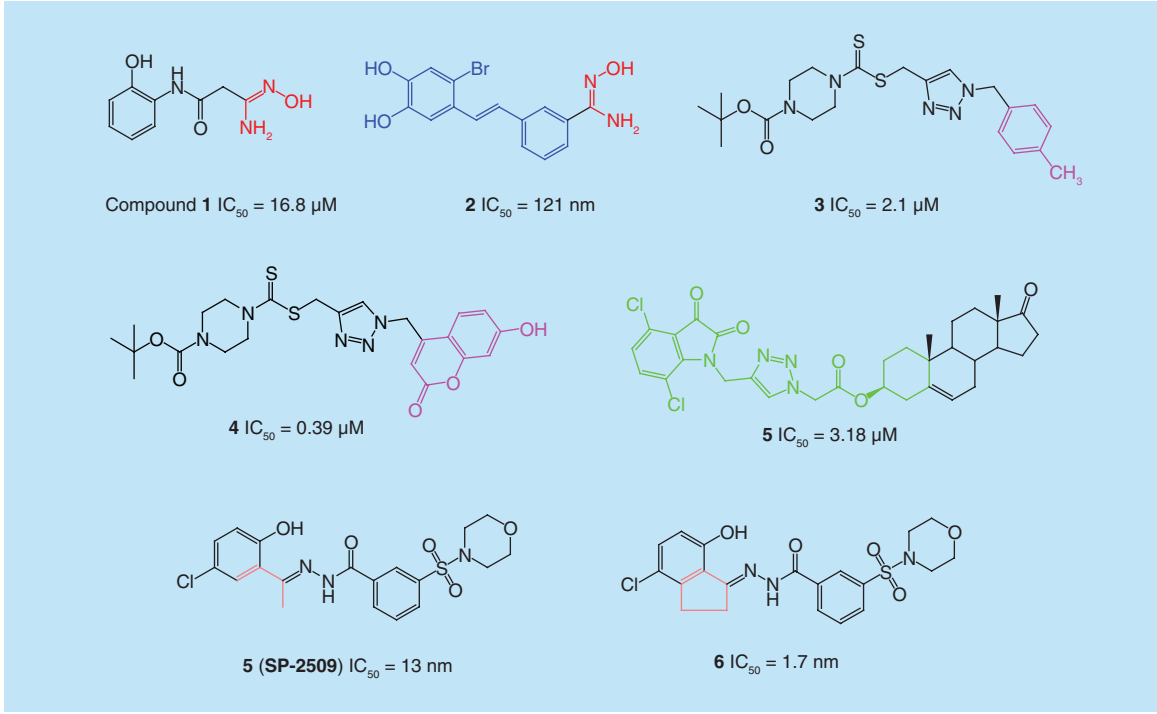

Figure 6. Recently reported representative lysine specific histone demethylase 1 inhibitors by our group and others.

showed significantly improved potency against LSD1 and high selectivity [51]. Compound **4** potently inhibited LSD1 (IC_{50} = 0.39 μM), 74-fold more potent than TCP. Of note, compound **4** showed high selectivity to LSD1 over MAOs (IC_{50} >1250 μM). Similarly, we identified the first steroid-based LSD1 inhibitor **5** using the above-mentioned strategy, which inhibited LSD1 with an IC_{50} value of 3.18 μM and cell growth of LSD1 overexpressed SH-SY5Y cells (IC_{50} = 4.06 μM), as well as induced G2/M cell cycle arrest and apoptosis [52,53]. SARs studies showed that the steroid nucleus of compound **5** was crucial for the activity, the compound (highlighted in green in compound **5**) without the steroid nucleus was inactive against LSD1. Docking simulations revealed that the steroid nucleus occupied the tubular hydrophobic cavity of the active site. Through the structure-based virtual screening of 13 million compounds, the Sharma group identified the *N′*-(1-phenylethylidene)-benzohydrazides as moderate LSD1 inhibitors [54], further optimizations led to the identification of SP-2509 (compound **5**, IC_{50} = 13 nM) as a reversible and noncompetitive LSD1 inhibitor. SP-2509 showed high selectivity to LSD1 over MAOs (IC_{50} > 300 μM) and inhibited survival of a panel of cancer cells at low micromolar levels with minimal inhibition against Cytochrome P450 (CYPs) and *hERG*.. Based on the structure of SP-2509, the Zhao group designed a new series of analogs using the conformational constraint strategy [55]. Among this series, compound **6** (IC_{50} = 1.7 nM) exhibited about eightfold increase in LSD1 inhibition, increased the methylation levels of H3K4me2 and H3K9me2 in A2780 cells and inhibited growth of LSD1 overexpressed cell lines at low micromolar levels.

Another series of LSD1 inhibitors highlighted here are compounds featuring a fused heterocycle (highlighted in red in Figure 7) installed with a basic amine group (highlighted in blue in Figure 7), which have been reported to occupy the substrate-binding region, thereby hindering the contact of H3 peptide substrate with FAD. The electrostatic interactions between the positively charged amine group and the negatively charged residues in the active site are crucial for the LSD1 inhibition. In 2015, our group reported a series of biaryl compounds installed with the thiourea group, which inhibited LSD1 at low micromolar levels [56]. Compound **7** inactivated LSD1 with an IC_{50} of 650 nM and was selective to LSD1 over MAOs (IC_{50} >1250 μM). Further mechanistic studies showed compound **7** inhibited cell migration and evasion, induced apoptosis and robustly suppressed growth of MGC-803 cells overexpressing LSD1 *in vivo* without significant toxicity. Following this work, our group recently performed extensive SARs studies using the scaffold hopping and bioisosteric replacement strategies, leading to the discovery of new LSD1 inhibitors **8** and **9**, which reversibly inhibited LSD1 with the IC_{50} values of 154 and 564 nM [57,58]. Compound **8** concentration-dependently inhibited migration of A549 and PC-9

cells, but exerted different effects on the expression levels of E-cadherin and N-cadherin. Compound **9** showed good selectivity to LSD1 over MAOs (inhibitory rate for MAO-A/B is 59 and 39%, respectively at 10 μM) and suppressed cell migration of MGC-803 cells and increased accumulation of H3K4me1/2, H3K9me1/2 and CD86, confirming its cellular LSD1 inhibition. Based on the TCP scaffold, Song *et al.* rationally designed a series of 3-(piperidin-4-ylmethoxy)pyridine containing compounds as potent LSD1 inhibitors [59]. Among this series, compound **10** (also known as **GSK-354**) inactivated LSD1 with the K_i value of 29 nM, exhibited high selectivity to LSD1 over MAOs (IC_{50} = >50 and 18.7 μM, respectively for MAO-A and MAO-B), increased cellular H3K4 methylation and robustly suppressed proliferation of AML and solid tumor cells. In 2016, the Incyte Corporation filed a series of patents on LSD1 inhibitors, which are structurally similar to GSK-354 differing in the core structures. Such series of compounds (e.g., compounds **11–15**) showed potent inhibition against LSD1 [60,61,62,63].

Similar to structures in Figure 7, the quinazoline-derived compound **E11** equipped with a hydrophilic amine group (compound **16** in Figure 8A), originally identified as the histone H3K9 methyltransferases G9a and G9a-like inhibitor, was recently reported to be able to inhibit LSD1 moderately (K_d = 243 nM) [28]. **E11** obstructed the entrance of the active site through forming unique multiple π-stacking interactions (Figure 8C), distant from the FAD cofactor (Figure 8B). As shown in Figure 8D, the negatively charged residues (highlighted in red) represent the primary binding region and form the electrostatic interactions with the dimethylamine group of **E11**. Of note, the protein surface area highlighted in red in Figure 8D is highly negatively charged and can serve as an attractive region for designing reversible LSD1 inhibitors. Above studies suggest that fused heterocyclic scaffolds installed with a hydrophilic amine group may represent an attractive structural template for designing new LSD1 inhibitors.

Very recently, Vianello *et al.* initiated a high-throughput screening (HTS) campaign using a time-resolved fluorescence resonance energy transfer technology to identify new reversible LSD1 inhibitors (Figure 9) [64,65]. The initial HTS of compound collection containing 34,000 small molecules using the time-resolved fluorescence resonance energy transfer assay led to the discovery of 115 hit compounds, of which compound

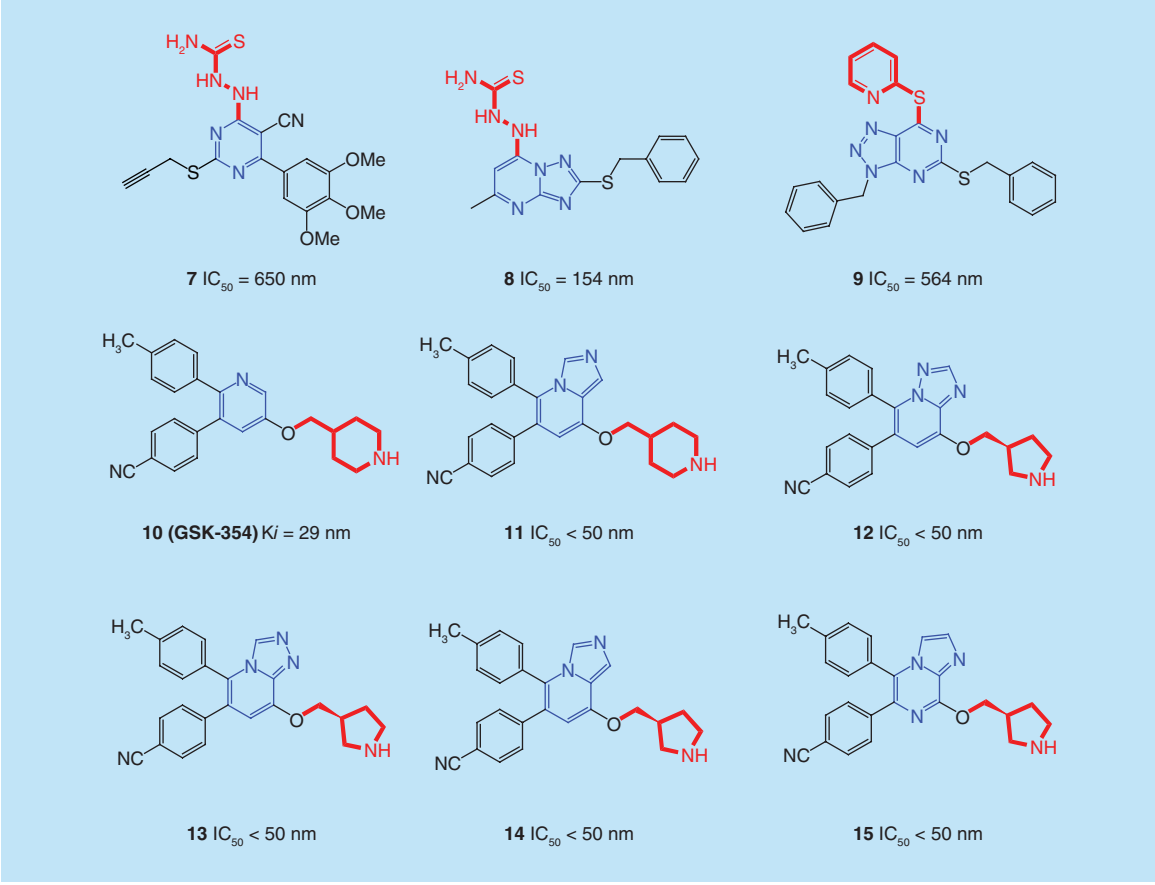

Figure 7. Representative fused heterocycles as lysine specific histone demethylase 1 inhibitors.

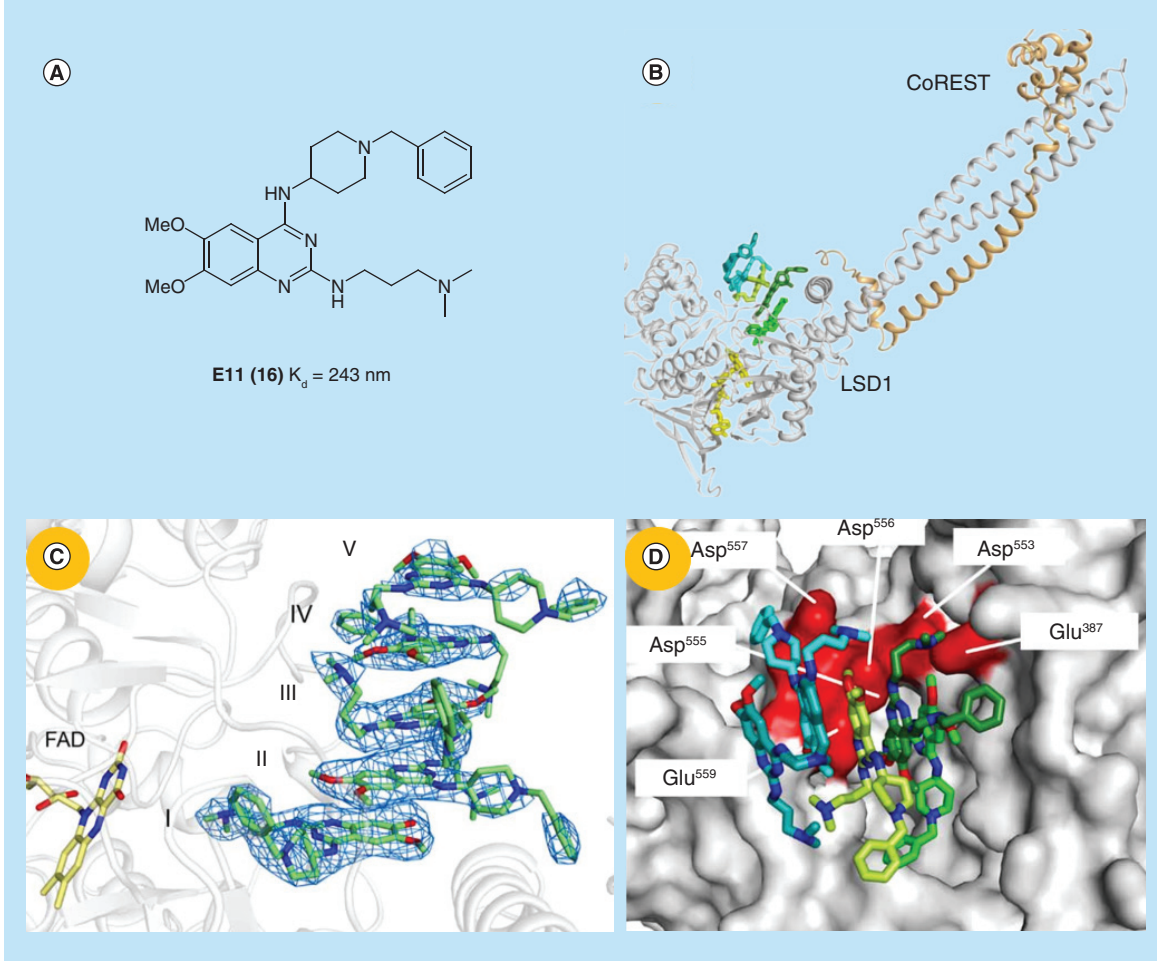

Figure 8. Noncovalent quinazoline-derived compound E11 occupied the active site of lysine specific histone demethylase 1 by forming unique π-stacking interactions. (A) Chemical structure of **E11**; **(B) E11** (green sticks) binding the substrate-binding site of LSD1-CoREST (white and wheat cartoon, respectively) at >5 Å from FAD (yellow sticks); **(C)** A stack of five molecule inhibitors (green sticks) bind at the entrance of the active site of the LSD1-CoREST complex; **(D)** Surface maps of negatively charged residues (red) of LSD1.
CoREST: RE1-Silencing transcriptional corepressor 1; FAD: flavin adenine dinucleotide; LASD1: Lysine specific histone demethylase 1.
(B–D) Adapted with permission from [28] © AAAS (2016).
For color figures, please see online at: https://www.future-science.com/doi/full/10.4155/fmc-2017-0068

17 was prioritized for its acceptable biochemical data and high-potential derivatization. Importantly, the compound **17** was successfully crystallized in complex with LSD1/CoREST. The cocrystal structure of compound **17**-LSD1/CoREST complex provided a fundamental structural basis for structure-based design of LSD1 inhibitors. As shown in Figure 9, compound **17** is buried into the hydrophobic catalytic site of LSD1, while two electro-negative regions at the exit vector (highlighted in red) are unoccupied, suggesting that additional interactions at these two sites may improve the potency. Further SARs studies focusing on the variations of the heterocycle, ring substitution as well as amide and phenyl replacement led to the discovery of potent LSD1 inhibitor **18** (IC_{50} = 162 μM) probably due to the interaction of the positively charged piperidine tail with the negatively charged site Asp375 or Asp556. While compound **18** exhibited two possible binding poses, the interaction with Asp375 (highlighted in green) was favored in terms of energies, another binding pose (highlighted in pink) pointing toward an interesting cluster of residues Asp555 and Asp556 adopted a less favored U-shaped conformation. The structural analysis of ligand (structure not shown here)-LSD1/CoREST indicated that *ortho* substituted compound accommodated the benzamide moiety, favoring aromatic–aromatic interactions and directed the basic terminal group toward Asp555. Compound **19** displayed extremely high potency against LSD1 (IC_{50} = 7.8 nM) and high selectivity to LSD1 over LSD2 and MAO-A/B (IC_{50} = 12.9,

41.3 and >100 μM, respectively). The ethyloxymethyl chain formed interaction with Gln358, which was responsible for the conformational restraints on the *ortho* longer chain and further induced the U-shaped conformation. Compound **19** inhibited 70% of colony formation at 1 μM in THP-1 cells, transcriptionally affected expression of *CD14, CD11b* and *CD86* genes, and showed significant anticlonogenic effect on MLL-AF9 cells. The thieno[3,2-b]pyrrole scaffold may represent a privileged core structure for designing new reversible LSD1 inhibitors. Of particular interest are those several co-crystal structures of small-molecule ligand-LSD1/CoREST complexes reported by Vianello *et al.* that reveal underexploited binding regions for designing new LSD1 inhibitors.

The rhodium (III) complex **20** (Figure 10A) was recently reported to be the first metal-based competitive LSD1 inhibitor (K_i = 0.57 μM), which inhibited LSD1 with an IC_{50} value of 40 nM (Figure 10B) and showed high selectivity to LSD2, KDM7 and MAOs (little or no inhibition was observed in PC-3 cells) [66]. Docking studies showed that the complex **20** occupied almost the entire substrate-binding site of LSD1 (PDB id: 2V1D, Figure 10C), forming hydrogen interactions with negatively charged residues Asp375 and Asp556. Complex **20** downregulated GLUT1 expression, suppressed H3K4me2 demethylation accompanying by increased amplification of p21, FOXA2 and BMP2, and potently inhibited growth of PC-3 cells (IC_{50} = 2.83 μM). Collectively, complex **20** could be considered as a potential scaffold for designing potent metal-based LSD1 inhibitors for the treatment of prostate cancer.

Conclusion & future perspective

The biological roles of LSD1 have been extensively explored since its identification in 2004, showing that LSD1 is involved in diverse biological process and its dysfunction is associated with the development of different types of diseases. Of note, aberrant overexpression of LSD1 has been observed in various human

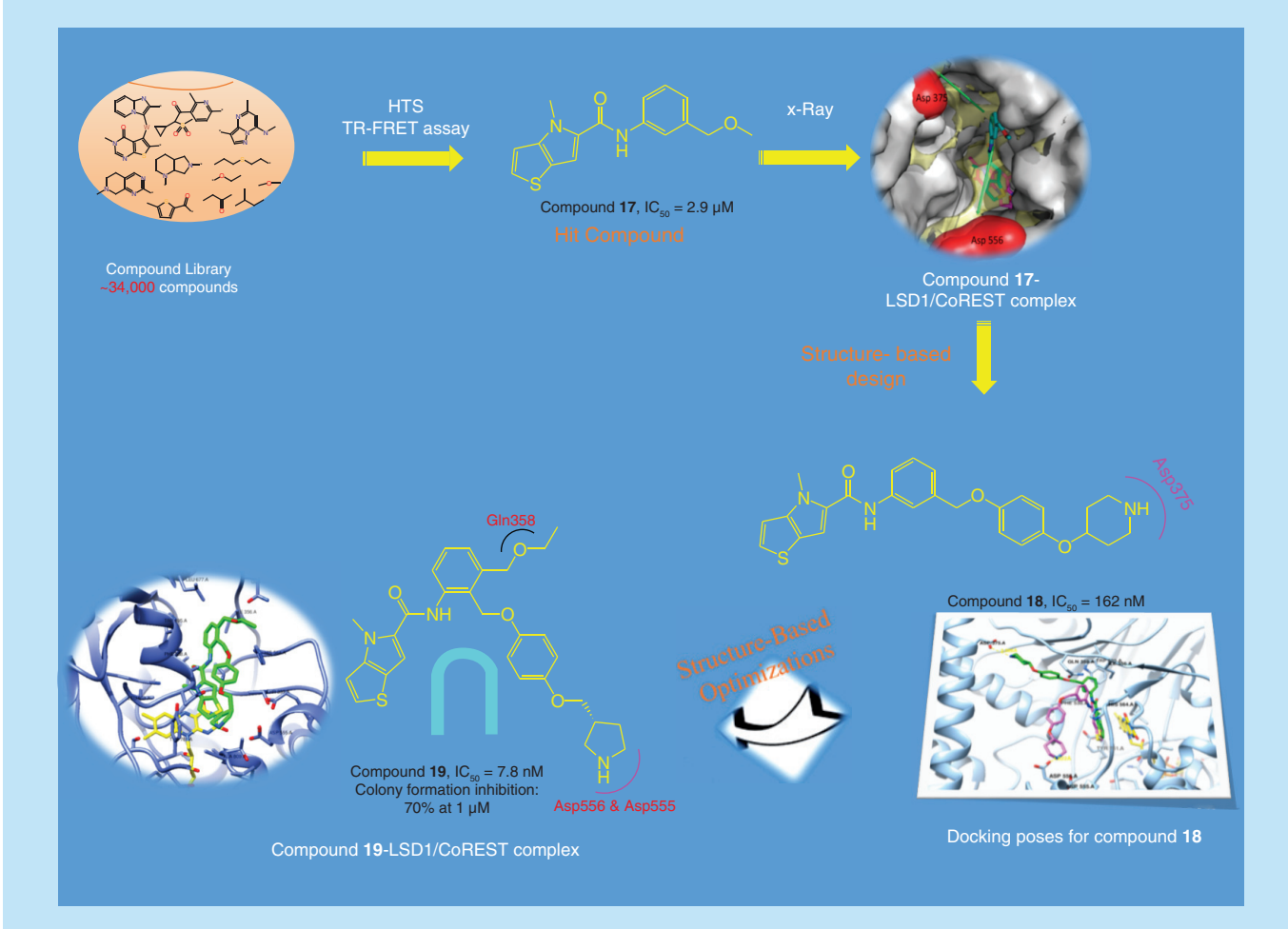

Figure 9. Structure-based rational design of new U-shaped thieno[3,2-b]pyrroles as highly potent lysine specific histone demethylase 1 inhibitors.

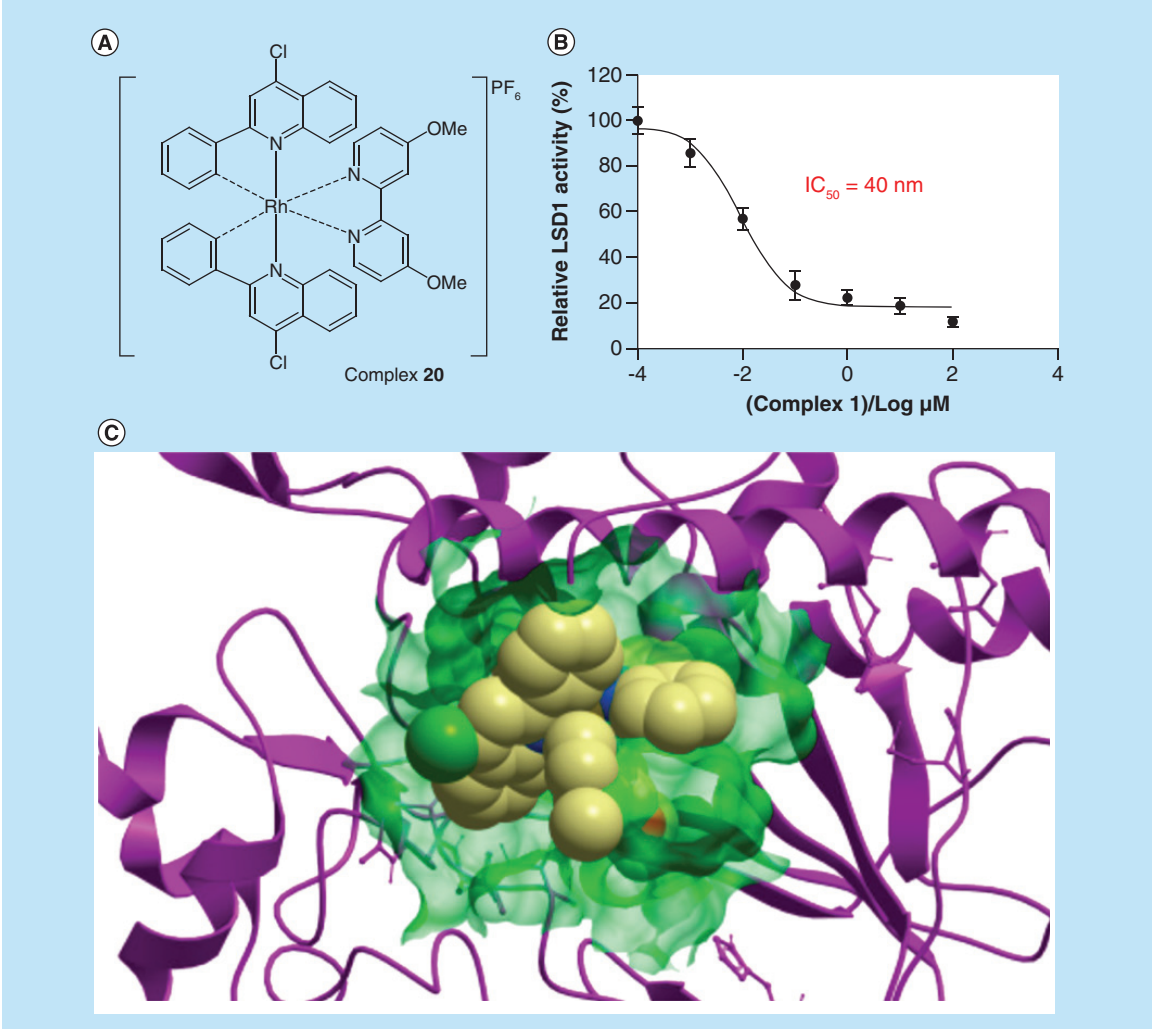

Figure 10. The first rhodium(III)-based lysine specific histone demethylase 1 inhibitor. (A) Chemical structure of complex **20**; **(B)** complex **20** concentration-dependently inhibits LSD1 demethylase activity. **(C)** Binding pose of complex **20** (yellow) in the active site (green surface) of LSD1.
(B & C) Reprinted with permission from [66] © American Chemical Society (2017).

cancer cells, and inactivation by small molecules or RNAi-mediated downregulation inhibits cancer cell differentiation, proliferation, invasion and migration as well as tumor growth. LSD1 has become an important biologically validated epigenetic target for cancer therapy [67]. To date, a large number of LSD1 inhibitors with different chemotypes have been reported, some of which (RG6016, GSK-2879552, INCB059872, etc.) have advanced into clinical trials for the treatment of AML, SCLC, etc. However, all these compounds in clinical trials are the mechanism-based FAD-dependent irreversible LSD1 inhibitors, none of reversible LSD1 inhibitors are currently undergoing clinical evaluation for cancer therapy. The design of highly potent and specific reversible LSD1 inhibitors for cancer therapy is still challenging, although several potent LSD1 inhibitors have been reported. Future work on the development of LSD1 inhibitors may take the following into account:

- The unfunctionalized nature of the substrate-binding region makes the design of potent reversible LSD1 inhibitors still challenging, though several potent LSD1 inhibitors targeting this region have been reported in last 3 years. Vianello *et al.* recently revealed new binding sites within the substrate-binding region and designed highly potent reversible LSD1 inhibitors (Figure 9) [28]. Therefore, revealment of underexploited binding sites in the substrate-binding region may facilitate the development of new potent and specific LSD1 inhibitors. Additionally, the H3 binding pocket, as an allosteric site, is able to regulate rotation of the amine oxidase domain with respect to the

TOWER domain, thereby affecting the overall receptor flexibility [68]. Targeting H3 pocket is capable of reducing LSD1 amino oxidase activity, competitively blocking the binding of transcription factors and preventing anchoring of chromatin to LSD1/CoREST. However, no allosteric LSD1 inhibitors have been reported to date. Targeting H3 binding pocket will be an important strategy to design allosteric LSD1 inhibitors.

- The success of RG6016, GSK-2879552, INCB059872, etc. in clinical trials makes TCP a privileged scaffold for designing irreversible LSD1 inhibitors. The structural analysis of TCP-FAD adducts reveals that the phenyl ring of TCP is a potential modifiable position for designing new LSD1 inhibitors. Especially, the introduction of basic moiety to the phenyl ring may improve the potency through targeting the negatively charged regions. The fused heterocycles equipped with a basic group are a class of emerging scaffolds for designing new reversible LSD1 inhibitors with unusual binding models. Compound **E11** in Figure 8 adopted unique multiple π-stacking interactions at the entrance of the active site. Additionally, NPs will be a rich source for searching LSD1 inhibitors. A few of NPs have been proved to be able to inhibit LSD1. The metal-based complexes will serve as new templates for designing novel LSD1 inhibitors.
- LSD1 shares 20% sequence similarity with MAOs in the highly conserved AOL region, and the AOL region of LSD1 is relatively larger than that of MAOs. Therefore, the MAOs inhibitors could be used as templates to rationally design new LSD1 inhibitors based on the structural features between LSD1 and MAOs.
- LSD1 inhibitors in combination with other therapeutic agents for cancer therapy have witnessed success in recent years and will be an important therapeutic strategy for cancers. As shown in Table 1, TCP/ATRA, TCP/ATRA/cytarabine and GSK-2879552/azacitidine combinations are currently undergoing clinical assessment for the treatment of AML and MDS. Recent evidence has showed that the combination of LSD1 inhibitors and pan-HDAC inhibitors is highly effective against human AML cells [69].
- Removal of PAINS compounds from compound collections. HTS and structure-based virtual screening have been successfully used to identify LSD1 inhibitors, for example, SP-2509. In this respect, particular attention should be paid to PAINS compounds (pan-assay interference compounds), which should be removed from the molecular library prior to the biological testing to avoid unnecessary work.

Executive summary

TCP as a promising scaffold for identifying new lysine specific histone demethylase 1 inhibitors

- LSD1 has been recognized as an important epigenetic target for cancer therapy, several irreversible LSD1 inhibitors RG6016, GSK-2879552, INCB059872, etc. have advanced into clinical trials for the treatment of AML, SCLC, etc.
- Tranylcypromine (TCP) is a privileged scaffold for designing mechanism-based FAD-dependent irreversible LSD1 inhibitors and will continue find its applications in this field.

Structure-based design of LSD1 inhibitors

- It would be a viable strategy to rationally design new LSD1 inhibitors based on the reported structures of MAOs inhibitors.
- The design of small molecules interrupting the stabilized conformation of the LSD1-bound H3 peptide could be viable strategy for designing LSD1 inhibitors.
- The search of reversible LSD1 inhibitors is still challenging, the discovery of underexploited binding sites within the substrate-binding region may facilitate the design of new reversible LSD1 inhibitors.
- Targeting H3 binding pocket would be a viable strategy to design allosteric LSD1 inhibitors.

Fused heterocycles equipped with a terminal basic group: emerging scaffolds of LSD1 inhibitors

- Fused heterocycles installed with a terminal basic group and metal-based complexes will serve as new scaffolds for designing LSD1 inhibitors.

Natural LSD1 inhibitors

- Natural products will be rich sources for identifying new LSD1 inactivators. Thiol-reactive compounds such as flavones and chalcones are potential LSD1 inhibitors.

PAINS compounds

- PAINS compounds always cause nonspecific binding with many enzymes and therefore should be removed from compound collections to avoid false-positive effects when performing high-throughput screening and structure-based virtual screening.

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