ACS Medicinal Chemistry Letters

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Discovery of Pyrrolo[2,3-*c*]pyridines as Potent and Reversible LSD1 Inhibitors

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Cite This: https://doi.org/10.1021/acsmedchemlett.3c00292 Read Online

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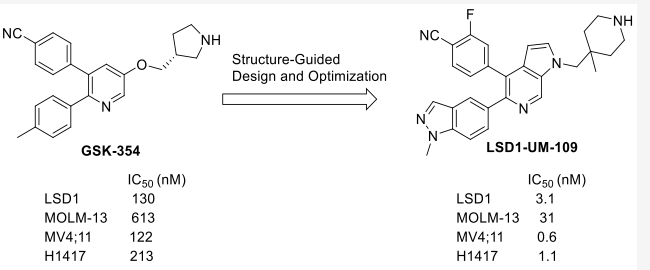
ABSTRACT: Lysine specific demethylase 1 (LSD1) acts as an epigenetic eraser by specifically demethylating mono- and histone 3 lysine 4 (H3K4) and H3 lysine 9 (H3K9) residues. LSD1 has been pursued as a promising therapeutic target for the treatment of human cancer, and a number of LSD1 inhibitors have been advanced into clinical development. In the present study, we describe our discovery of pyrrolo[2,3-*c*]pyridines as a new class of highly potent and reversible LSD1 inhibitors, designed on the basis of a previously reported LSD1 inhibitor GSK-354. Among them, **46** shows an IC_{50} value of 3.1 nM in inhibition of LSD1 enzymatic activity and inhibits cell growth with IC_{50} values of 0.6 nM in the MV4;11 acute leukemia cell line and 1.1 nM in the H1417 small-cell lung cancer cell line. Compound **46** (LSD1-UM-109) is a novel, highly potent, and reversible LSD1 inhibitor and serves as a promising lead compound for further optimization.



KEYWORDS: *LSD1, structure-guided design, novel inhibitors, histone demethylase, acute leukemia, small-cell lung cancer*

Histone methylation is one of the most important post-translational modifications and plays an essential role in the regulation of gene expression. Lysine specific demethylase 1 (LSD1), also known as lysine (K)-specific demethylase 1A (KDM1A), is a flavin-dependent monoamine oxidase, which can demethylate mono- and dimethylated lysines, specifically histone 3, lysines 4 and 9 (H3K4 and H3K9) residues. By functioning as an epigenetic eraser, LSD1 regulates gene expression,[1] and plays a critical role in many processes such as tumorigenesis, stem cell biology, neurodegenerative disorders, viral infection, diabetes, and fibrosis.[2−8] Dysregulation of LSD1 is tightly associated with the progression of human cancers, and inhibition of LSD1 by either RNAi or small molecules blocking cell proliferation, malignant transformation, and epithelial mesenchymal transition (EMT) process of tumor cells.[9−12] Furthermore, inhibition or depletion of LSD1 stimulates antitumor immunity and enhances the antitumor efficacy of immune checkpoint blockade.[13,14] Hence, LSD1 inhibition is potentially a promising new cancer therapeutic strategy.[15,16]

In the past decade, extensive research efforts have been devoted to the discovery of LSD1 inhibitors, which are broadly divided into reversible and irreversible inhibitors based upon their mode of action (Figure 1). The most well-studied irreversible inhibitors contain a cyclopropylamine moiety that forms a covalent bond with the cofactor FAD for LSD1. To date, five such irreversible LSD1 inhibitors have been advanced to human clinical trials. ORY-1001 (**1**) (Clinicaltrials.gov identifier NCT02913443) from Oryzon Genomics is the first and potent irreversible LSD1 inhibitor advanced into clinical development, is currently in phase 2 trials for acute myeloid leukemia (AML) and small cell lung cancer (SCLC).[17−19] Another inhibitor, ORY-2001 (**2**) (Clinicaltrials.gov identifier NCT03867253) is a CNS optimized, dual LSD1/MAO-B inhibitor currently in phase II clinical trials for the treatment of Alzheimer's disease. GSK2879552 (**3**) (Clinicaltrials.gov identifier NCT02177812), IMG-7289 (**4**) (Clinicaltrials.gov identifier NCT03136185), and INCB59872 (**5**) (Clinicaltrials.gov identifier NCT02712905) have also been advanced into clinical development.[20−22] However, tranylcypromine (TCP)-based LSD1 inhibitors are often accompanied by side effects at least in part due to the covalent binding to FAD. Therefore, noncovalent and reversible LSD1 inhibitors have been sought.[17,23−26] Currently, two reversible LSD1 inhibitors, CC-90011 (**6**) (Clinicaltrials.gov identifier NCT02875223) from Celgene and SP-2577 (**7**) (Clinicaltrials.gov identifier NCT03895684) from Salarius Pharmaceuticals, have been advanced into clinical development.[27]

Received: July 5, 2023
Accepted: September 14, 2023

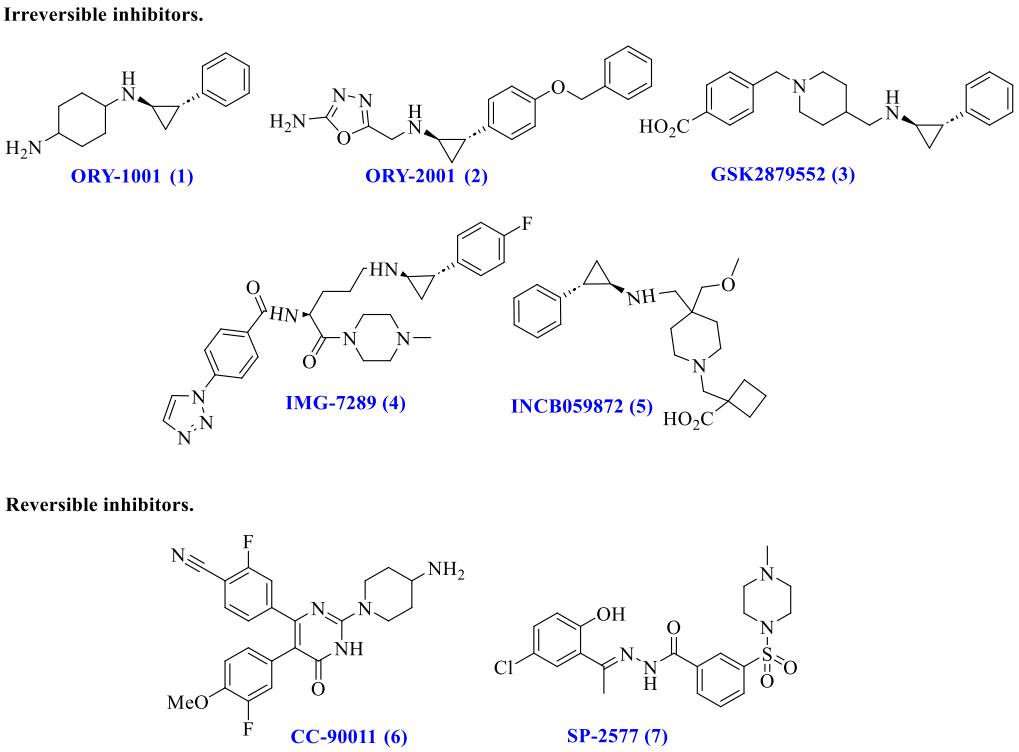


Figure 1. Chemical structures of irreversible and reversible LSD1 inhibitors advanced in human clinical trials.

LSD1 is crucial for maintaining cellular homeostasis in embryonic stem cells (ESCs) and adult hematopoiesis. Genetic deletion of LSD1 in murine ESCs has been shown to result in early death and knockdown of LSD1 in hematopoietic cells in adults can lead to pancytopenia.[28] Given these findings, we posited that reversible inhibition of LSD1 may achieve a better therapeutic index than the irreversible inhibition of LSD1 for the treatment of human cancer. Because no LSD1 inhibitor has been approved for marketing, the discovery of potent reversible LSD1 inhibitors with novel chemical scaffolds is still of considerable value for the ultimate success in targeting LSD1 as a new therapeutic strategy for the treatment of human cancers and potentially other human diseases. In the present study, we describe our efforts in the design, synthesis, and evaluation of a new class of highly potent, reversible LSD1 inhibitors.

We started our design from GSK-354 (**8**, Figure 2A), the first reported reversible inhibitor, which exhibited good potency in biochemical and cellular assays.[20] To guide our design, we modeled the structure of GSK-354 in a complex with LSD1 based upon the cocrystal structure of the PKSFLV peptide complexed with human LSD1 (PDB ID: 3ZMU)[29] (Figure 2C). Our predicted binding model suggested that the tolyl, benzonitrile, and pyrrolidine methyl groups on the pyridine scaffold in GSK-354 project into the three substrate-binding pockets of the LSD1. Specifically, the cyano group of GSK-354 interacts with K661 and is close to the cofactor, FAD, the pyrrolidine group forms a hydrogen bond with the backbone carbonyl group of W552 and a salt bridge with the side chain of D555, and the tolyl group of GSK-354 docks into a hydrophobic pocket close to the entrance of the binding site (Figure 2C).

Based on our predicted binding model, additional space is available close to the pyridine moiety of GSK-354 (**8**). We

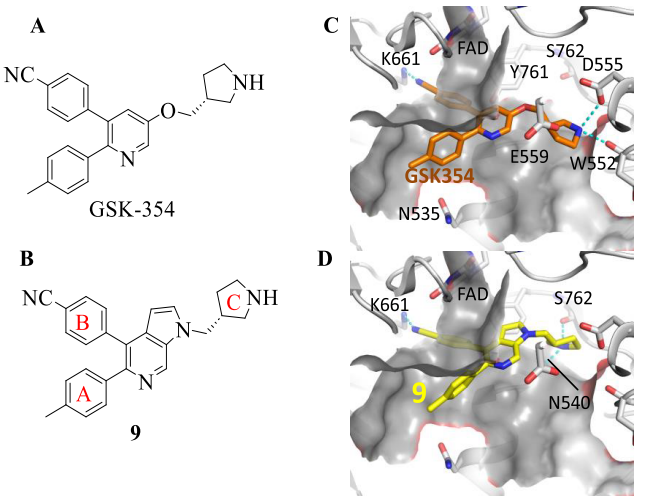


Figure 2. (A, B) Chemical structures of GSK-354 (**8**) and our designed LSD1 inhibitor (**9**). (C, D) Predicted binding models of GSK-354 and compound **9** in a complex with LSD1 using the cocrystal structure PDB ID: 3ZMU as the template.

replaced the pyridine ring with a bicyclic 1*H*-pyrrolo[2,3-*c*] pyridine, which led to the design of compound **9** (Figure 2D). Our modeling showed that compound **9** and GSK-354 bind to LSD1 similarly but the additional fused ring in compound **9** captures additional hydrophobic interactions with the Tyr761 residue.

We synthesized and evaluated compound **9** for its inhibition of the LSD1 enzymatic activity. Compound **9** achieved IC_{50} = 80 nM and was thus slightly more potent than GSK-354 in the same assay (Table 1). Encouraged by its reasonably potent LSD1 inhibition activity, we performed extensive modifications

https://doi.org/10.1021/acsmedchemlett.3c00292
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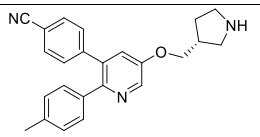
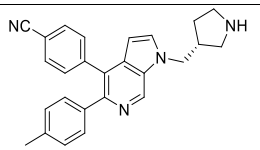
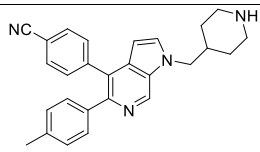
on the three rings in compound **9** (labeled as A−C, Figure 2B).

Table 1. Design of New LSD1 Inhibitors Based upon GSK-354 (8)

Compound No.	Structures	IC_{50} (nM)[a]
GSK-354 (**8**)		130 ± 40[b]
9		80 ± 43
10		34.5 ± 7.3

[a]IC_{50} values were determined by an AlphaLISA assay; values reported are the mean ± SD of three experiments. [b]Values reported are the mean ± SD of five experiments.

We first replaced the pyrrolidine group in compound **9** with a piperidine group, which resulted in compound **10**. Compound **10** has an IC_{50} value of 34.5 nM in inhibiting LSD1 enzymatic activity (Table 1) and is thus 2-times better than compound **9**.

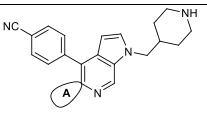
Because compound **10** is more potent than compound **9** and has no chiral center, we decided to use compound **10** as the new template molecule for further modifications.

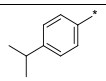
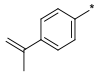
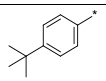
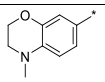
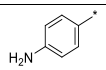
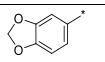
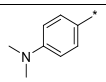
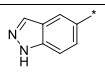
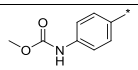
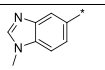
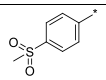
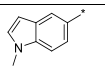
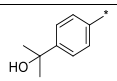
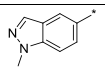
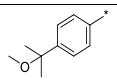
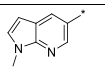
We replaced the 4-methyl group in ring A with several bulkier hydrophobic groups and obtained compounds **11**, **12**, and **13** (Table 2). These compounds showed inhibitory potencies of LSD1 similar to those of compound **10**. The inhibition data for compounds **10**-**13** are consistent with our predicted binding models for GSK-354 and compound **9**, which showed that the corresponding 4-methyl group is exposed to a solvent environment. We next probed this site by replacing the 4-methyl group with hydrophilic groups.

Replacement of the methyl group with an amino group led to compound **14**, which inhibits LSD1 with an IC_{50} value of 354 nM and is thus 10-fold less potent than **10**. Surprisingly but gratifyingly, dimethyl substitution of the amino group in **14** yielded compound **15**, which inhibits LSD1 with an IC_{50} value of 9.4 nM. Compound **15** is thus >35-times more potent than **14** and 4-times more potent than **10**, representing a potent LSD1 inhibitor.

Encouraged by the potent LSD1 inhibitory activity for **15**, we made additional analogues with a hydrophilic substituent at the 4-position of the phenyl ring. Compound **16**, containing 4-methylcarbamate, has an IC_{50} value of 53.8 nM in inhibition of LSD1. Compound **17**, with a 4-methylsulfonyl, is 5-times less potent than **10**. Compound **18**, with a 2-hydroxypropan-2-yl, inhibits LSD1 with an IC_{50} value of 21.1 nM, and compound

Table 2. SAR of Aryl Modifications of Ring A of Compound 10



No.	A	IC_{50} (nM)[a]	No.	A	IC_{50} (nM)[a]
10		34.5±7.3	**20**		24.3±5.4
11		46.1±17.8	**21**		69.8±26.7
12		58.5±14.8	**22**		> 1000
13		47.9±20.0	**23**		4.6±0.7
14		354±88	**24**		20.5±3.0
15		9.4±2.5	**25**		94.3±45.5
16		53.8±9.3	**26**		15.4±5.9
17		146±47	**27**		6.5±0.6
18		21.1±2.0	**28**		12.9±4.3
19		16.5±4.0	**29**		5.1±1.6

[a]IC_{50} values were determined by an AlphaLISA assay; values reported are the mean ± SD of three independent experiments.

19, with a 2-methoxypropan-2-yl inhibits LSD1 with an IC_{50} value of 16.5 nM.

We installed an additional methyl group in the 3-position on the phenyl ring of **10** and obtained compound **20**, which is slightly more potent than **10**. Changing the 4-methyl phenyl in **10** into a pyridine yielded compound **21**, which is 2-times less potent than **10**. Changing the 4-methyl phenyl with 1-methyl-1*H*-pyrazole resulted in compound **22**, which is >20-fold less potent than **10**.

We synthesized compound **23** by cyclization of the *N*-methyl group with the phenyl in compound **15** to form a fused 6-membered ring. Compound **23** inhibits LSD1 with an IC_{50} value of 4.6 nM and is thus 2-times more potent than **15**.

Encouraged by the excellent inhibitory potency of **23**, we synthesized a series of compounds containing a bicyclic ring system and obtained compounds **24**-**29**. Among them, compound **27**, containing 1-methyl-1*H*-indole, and compound **29**, containing 1-methyl-1*H*-pyrrolo[2,3-*b*] pyridine, achieved IC_{50} values of 6.5 and 5.1 nM, respectively. Hence, our modifications of ring A have identified compounds **23**, **27**, **28**,

https://doi.org/10.1021/acsmedchemlett.3c00292
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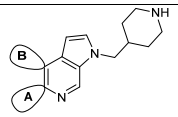
and **29** as potent LSD1 inhibitors with single digit nanomolar IC_{50} value.

Next, we performed further optimization of the phenyl ring B in compounds **23** and **28** (Table 3). Installation of the 2-F or

Table 3. SAR of Aryl Modifications of the Phenyl Ring B on the Pyrrolo Pyrimidine Scaffold



No	A	B	IC_{50} (nM)[a]
28			12.9±4.3
30			3.9±0.9
31			17.5±4.9
32			156±14
33			215±98
34			6250±529
23			4.6±0.7
35			4.3±1.1

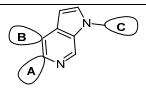
[a]IC_{50} values were determined by an AlphaLISA assay; values reported are the mean ± SD of three experiments.

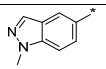
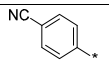
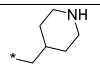
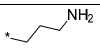
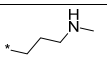
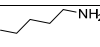
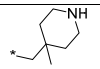
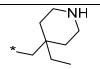
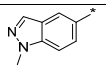
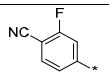
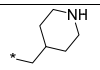
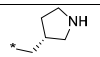
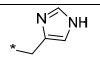
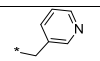
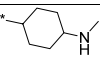
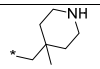
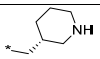
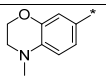
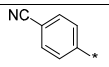
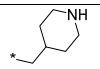
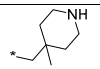
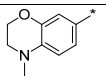
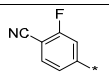
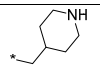
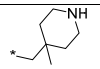
3-F substituent in compound **28** resulted in compounds **30** and **31**, respectively. While compound **31** is slightly less potent than compound **28**, compound **30** inhibits LSD1 with an IC_{50} value of 3.9 nM and is 3-times more potent than **28**. Installation of 2-CH_3 or 3-CH_3 substituent in compound **28** resulted in compounds **32** and **33**, respectively, but both compounds are >10-times less potent than **28** in inhibition of LSD1. Changing the nitrile into an amide yielded compound **34**, which is >50-times less potent than **28**. Installation of 2-F substitution on the phenyl ring B in **23** yielded compound **35**, which has a similar inhibitory potency of LSD1 as compared to compound **23**.

We next performed extensive modifications of ring C using compounds **23**, **28**, and **30** as the template molecules, with the results summarized in Table 4.

Replacement of ring C in compound **28** with a hydrogen atom resulted in compound **36**, which has an IC_{50} value of 3.7 μM and is thus >300-times less potent than **28**, highlighting the importance of ring C for achieving high potency in inhibition of LSD1. We synthesized compounds **37** and **38** using linear primary or secondary amine group. Compound **37**, containing a propylamine, has an IC_{50} value of 28.4 nM and is 2-times less potent than **28**. Replacement of the primary amine in **37** with a secondary amine yielded compound **38**, which was 2-times less potent than **37**. Replacing the propylamine in **37**

Table 4. Optimization of Ring C



Compd No	A	B	C	IC_{50} (nM)[a]
28				12.9±4.3
36			H	3713±1625
37				28.4±9.3
38				42.8±4.6
39				12.4±3.0
40				4.3±1.6
41				121±29
30				3.9±0.9
42				3.7±0.9
43				>10000
44				>10000
45				229±32
46 (LSD1-UM-109)				3.1±1.1[b]
47				3.5±1.2
23				4.6±0.7
48				1.3±0.4
35				4.3±1.1
49				1.4±0.5
SP-2577 (7)				26.2[c]

[a]IC_{50} values were determined by an AlphaLISA assay; values reported are the mean ± SD of three experiments. [b]Values reported are the mean ± SD of five experiments. [c]Values reported are the mean ± SD of two experiments.

https://doi.org/10.1021/acsmedchemlett.3c00292
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with a butylamine group led to **39**, which is 2-times more potent than **37** and equally potent as **28**. Installation of a bridged methyl group onto the piperidine group generated compound **40**, which has an IC_{50} value of 4.3 nM in inhibition of LSD1 and is 3-times more potent than **28**. However, changing the bridged methyl group in **40** with an ethyl group afforded **41**, which is >25-times less potent than **40**, suggesting limited space available at this site.

We performed further modifications of ring C using compound **30** as the template molecule (Table 4). Compound **42** was obtained by replacing the piperidin-4-ylmethyl group in **30** with (*R*)-3-methylpyrrolidine, which has an IC_{50} value of 3.7 nM and is equally as potent as **30**. However, replacing the piperidin-4-ylmethyl group with 4-methyl-1*H*-imidazole or methyl-3-pyridine resulted in inactive compounds **43** and **44**, respectively, in the inhibition of LSD1. Replacement of the piperidin-4-ylmethyl group with *N*-methyl-4-cyclohexan-1-amine resulted in compounds **45**, which is 62-times less potent than **30**. Installation of a bridged methyl group onto the piperidin-4-ylmethyl group in **30** led to compound **46** (LSD1-UM-109), which has an IC_{50} value of 3.1 nM in inhibition of LSD1 and is slightly more potent than **30**. Changing the piperidin-4-ylmethyl group in **30** with (*R*)-3-methyl-1-piperidinyl afforded **47**, which has an IC_{50} value of 3.5 nM and is equipotent LSD1 inhibitor as **30**. We next synthesized compounds **48** and **49** by installing a methyl group onto the piperidin-4-ylmethyl group of compounds **23** and **35**, respectively. These compounds are highly potent LSD1 inhibitors with IC_{50} values of 1.3 and 1.4 nM, respectively.

SP-2577 (**7**) is a reversible LSD1 inhibitor currently in clinical development. We evaluated SP-2577 (**7**) in the LSD1 assay. Our data showed that SP-2577 (**7**) has an IC_{50} value of 26.2 nM. Hence, compound **46** is 8-times more potent than SP-2577 (**7**) in our assay.

LSD1 inhibitors have been shown to be effective in inhibiting cell growth in human AML and SCLC cell lines.[12] We next evaluated a number of our potent LSD1 inhibitors for their ability to inhibit cell growth in two representative AML cell lines and one representative SCLC cell line. Because LSD1 inhibitors block the demethylation activity of LSD1 on histone lysines and require chromatin remodeling to impact cell proliferation, a significant cell growth inhibitory effect was only achieved after a prolonged period of treatment (7−10 day treatment time).[12,30,31] Accordingly, we determined the cell growth inhibition activity for our LSD1 inhibitors in the MOLM-13 and MV4;11 AML cell lines with a 7 day treatment time and in the H1417 SCLC cell line with a 10 day treatment time. We included GSK-354 as a control compound in these experiments. The data are summarized in Table 5.

Our data (Table 5) showed that a number of these new LSD1 inhibitors potently inhibit cell growth in these three cell lines and are much more potent than GSK-354. Compound **46** achieves IC_{50} values of 31 0.6, and 1.1 nM in cell growth inhibition in MOLM-14, MV4;11 and H1417 cell lines, respectively, and is therefore a highly potent LSD1 inhibitor. In direct comparison, compound **46** is 20-, 201-, and 193-times more potent than GSK-354 in inhibiting cell growth in MOLM-14, MV4;11 and H1417 cell lines, respectively. Compound **49** achieves IC_{50} values of 182 nM, 0.7 nM and 2.3 nM in inhibition of cell growth in MOLM-14, MV4;11 and H1417 cell lines, respectively, and is also a potent LSD1 inhibitor. In direct comparison, compound **49** is 3-, 172-, and 92-times more potent than GSK-354 in inhibition of cell growth in MOLM-14, MV4;11 and H1417 cell lines, respectively. For all of the LSD1 inhibitors evaluated, the MV4;11 cell line is the most sensitive to LSD inhibitors, followed by the H1417 SCLC cell line, whereas the MOLM-13 cell line is the least sensitive. Nevertheless, a number of our LSD1 inhibitors are still capable of achieving IC_{50} values of <100 nM in the MOLM-13 cell lines.

Table 5. Inhibition of Cell Growth of LSD1 Inhibitors in Human Tumor Cell Lines

	IC_{50} ± SD		
no.	MOLM-13 (nM)[ab]	MV4;11 (nM)[ac]	H1417 (nM)[ac]
GSK-354 (**8**)	613 ± 365	122 ± 5	213 ± 103
23	170 ± 22	2.9 ± 1.8	9.3 ± 5.4
28	62 ± 36	4.1 ± 0.2	11.0 ± 7.0
30	316 ± 84	2.7 ± 0.5	7.3 ± 2.3
35	58 ± 26	1.7 ± 1.3	2.7 ± 0.9
40	126 ± 96	3.2 ± 1.5	5.7 ± 3.2
46	31 ± 7	0.6 ± 0.2	1.1 ± 0.5
48	130 ± 65	1.5 ± 0.6	3.7 ± 1.2
49	182 ± 21	0.7 ± 0.3	2.3 ± 0.7

[a]Mean of three experiments. [b]Cells were treated with compounds for 7 days. [c]Cells were treated with compounds for 10 days.

We next evaluated compounds **30**, **35**, **42**, **46**, and **49** for liver microsome stability, and the data are summarized in Table 6. Compounds **30**, **42**, and **46** have excellent microsomal stability in human, dog, rat, and mouse microsomes. In comparison, compounds **35** and **49** have moderate stability for rat and dog microsomes and poor stability in mouse and human microsomes.

Table 6. Liver Microsomal Stability of Representative LSD1 Inhibitors

	$T_{1/2}$ in liver microsomes (min)[a]			
no.	mouse	rat	dog	human
30	>60	>60	>60	>60
35	<10	42	37	<10
42	>60	>60	>60	>60
46	>60	>60	>60	>60
49	<10	36	35	<10

[a]$T_{1/2}$ half-life in liver microsome of different species.

In summary, in the present study, we have presented our design, synthesis, and evaluation of a series of pyrrolo[2,3-*c*] pyridines as new LSD1 inhibitors based upon GSK-354 (**8**) using a structure-guided approach. Our study identified compound **46** as a highly potent LSD1 inhibitor. Compound **46** inhibits LSD1 activity with IC_{50} values of 3.1 nM. Consistent with its potent LSD1 inhibitory activity, compound **46** (LSD1-UM-109) achieves IC_{50} values of 0.6, 31 nM in inhibition of cell growth in the MV4;11 and MOLM-13 AML cell lines and 1.1 nM in the H1417 SCLC cell line. Compound **46** displays excellent stability in mouse, rat, and human microsomes. Compound **46** is a potent and novel LSD1 inhibitor and a promising lead compound for further optimization toward the development of a highly potent and optimized LSD1 inhibitor for the treatment of human cancer and potentially other human diseases for which inhibition of LSD1 may have a benefit.

https://doi.org/10.1021/acsmedchemlett.3c00292
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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00292.

Chemical synthesis procedures, biological assay protocols, supporting figures, docking study protocol, compound characterization, and ^{1}H and ^{13}C NMR spectra of final compounds (PDF)

Molecular string files for all the final target compounds (XLS)

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Author Contributions

†C.Z. and R.K.R. contributed equally.

Notes

The authors declare the following competing financial interest(s): The University of Michigan has filed a patent application on these LSD1 inhibitors, which has been licensed by Ascentage Pharma Group. S. Wang, C. Zheng, R. Rej, and C.-Y. Yang are co-inventors on the patent application. The University of Michigan has received a research contract from Ascentage. S.W. is a co-founder of Ascentage, owns shares in Ascentage and is a paid consultant to Ascentage.

■ ACKNOWLEDGMENTS

This work was supported in part by a research contract from Ascentage Pharma Group (to S.W.), and the University of Michigan Rogel Cancer Center Support Grant from National Cancer Institute, NIH (P30 CA046592).

■ ABBREVIATIONS USED

AML, acute myeloid leukemia; ATRA, all-trans retinoic acid; BOC, *tert*-butyloxycarbonyl; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMA, dimethylacetamide; ESC, embryonic stem cell; EtOH, ethanol; K_d, dissociation constant; KDM1A, lysine (K)-specific demethylase 1A; LSD1, lysine specific demethylase 1; MeCN, acetonitrile; SPR, surface plasmon resonance; TFA, trifluoroacetic acid; TR-FRET, time-resolved fluorescence resonance energy transfer; FAD, flavin adenine dinucleotide; H3K4, histone H3 lysine 4; H3K4me1, -2, or -3, mono-, di-, or trimethylated H3K4; MAO, monoamine oxidase; MLL, mixed lineage leukemia; OTs, tosyl; SAR, structure−activity relationship; SCLC, Small Cell Lung Cancer; TCP, tranylcypromine

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https://doi.org/10.1021/acsmedchemlett.3c00292
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX