Journal of Medicinal Chemistry

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Perspective

# Tranylcypromine Based Lysine-Specific Demethylase 1 Inhibitor: Summary and Perspective

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Cite This: *J. Med. Chem.* 2020, 63, 14197−14215

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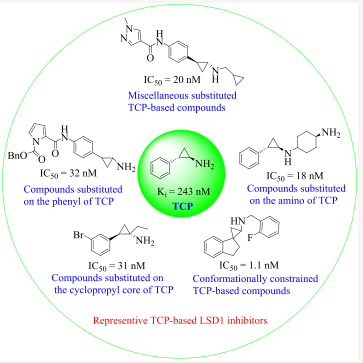
**ABSTRACT:** Histone lysine-specific demethylase 1 (LSD1/KDM1A) has become an important and promising anticancer target since it was first identified in 2004 and specially demethylates lysine residues of histone H3K4me1/2 and H3K9me1/2. LSD1 is ubiquitously overexpressed in diverse cancers, and abrogation of LSD1 results in inhibition of proliferation, invasion, and migration in cancer cells. Over the past decade, a number of biologically active small-molecule LSD1 inhibitors have been developed. To date, six *trans*-2-phenylcyclopropylamine (TCP)-based LSD1 inhibitors (including TCP, ORY-1001, GSK-2879552, INCB059872, IMG-7289, and ORY-2001) that covalently bind to the flavin adenine dinucleotide (FAD) within the LSD1 catalytic cavity have already entered into clinical trials. Here, we provide an overview about the structures, activities, and structure−activity relationship (SAR) of TCP-based LSD1 inhibitors that mainly covers the literature from 2008 to date. The opportunities, challenges, and future research directions in this emerging and promising field are also discussed.






## 1. INTRODUCTION

Among the diverse epigenetic modifications on histone, including acetylation, methylation, phosphorylation, ubiquitination, hydroxylation, sumoylation, and glycosylation,[1] histone methylation was originally considered as an irreversible process until histone lysine-specific demethylase 1 (LSD1/KDM1A) was identified by Shi's group in 2004.[2] LSD1 specifically erases the methyl groups from mono- and dimethylated histone 3 lysine 4 and lysine 9 by coordinating with CoREST in a flavin adenine dinucleotide (FAD)-dependent manner, thereby regulating target gene transcription by modulating the chromatin structure. In addition, LSD1 has also been reported to remove mono- and dimethyl groups from specific lysine residues on several non-histone proteins, such as DNA methyltransferase 1,[3] p53 (protein 53),[4] MYPT1 (myosin phosphatase targeting subunit 1),[5] E2F1 (E2F transcription factor 1),[6] STAT3 (signal transducer and activator of transcription 3),[7] and HIF1$\alpha$ (hypoxia-inducible factor 1$\alpha$),[8] which may lead to the dysregulation of their function.

LSD1 is overexpressed or dysregulated in various cancers, including gastric cancer, small cell lung cancer (SCLC), breast cancer,[9,10] neuroblastoma,[11] acute myeloid leukemia (AML), retinoblastoma,[12] and prostate cancer.[13−15] Pharmacological or genetic abrogation of LSD1 inhibits the proliferation and metastasis of diverse tumor cells.[16−19] Therefore, LSD1 has emerged as an attractive anticancer drug target, and numerous LSD1 inhibitors have been developed over the last ten years.[18,20−25] So far, six *trans*-2-phenylcyclopropylamine (TCP)-based LSD1 inhibitors, including TCP, ORY-1001,[26] GSK-2879552,[27,28] INCB059872,[29,30] IMG-7289, and ORY-2001, have reached the clinical trial stage alone or in combination with other therapeutics for the treatment of cancers and neurodegenerative disorders (Table 1).[16,20,31] Therefore, TCP has become an important druggable scaffold for developing potent LSD1 inhibitors.[16,32]

## 2. STRUCTURAL BASIS FOR DESIGNING NEW TCP-BASED LSD1 INHIBITORS

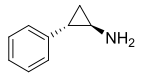
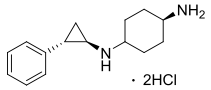
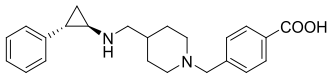
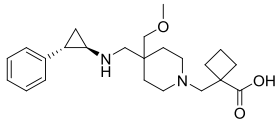
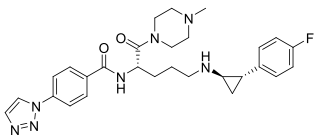
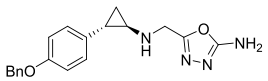
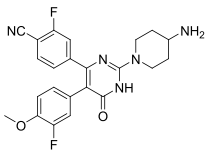
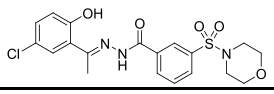
LSD1 demethylates H3K4me1/2 and H3K9me1/2 through amine oxidation using FAD as a cofactor. As shown in Figure 1A, with oxygen, methylated lysine suffers single-electron oxidation of the amine to form the corresponding iminium cation by LSD1 coupling with FAD, generating $FADH_2$ and $H_2O_2$. The resulting iminium cation intermediate is unstable and can easily be hydrolyzed by $H_2O$ to obtain the demethylated product and formaldehyde. Simultaneously, $FADH_2$ is reoxidized to FAD by consuming $O_2$, which forms $H_2O_2$. As illustrated in Figure 1B, TCP inactivates LSD1 through a single electron transfer mechanism, and then four different TCP-FAD adducts are obtained by the homolysis of the cyclopropyl ring through distinct pathways,[33−35] which is an irreversible catalytic process. First, FAD abstracts an

**Received:** May 30, 2020
**Published:** September 15, 2020




https://dx.doi.org/10.1021/acs.jmedchem.0c00919
*J. Med. Chem.* 2020, 63, 14197−14215

**Table 1. LSD1 Inhibitors in Clinical Trials**

| Drugs | Structure | Phase | Trial number | Diseases | Status |
|---|---|---|---|---|---|
| TCP |  | Phase I/II | NCT02261779, EudraCT 2012-002154-23 | Relapsed/refractory AML | Completed |
| | | Phase I/II | NCT02717884 | Non-M3 AML | Recruiting |
| | | Phase I | NCT02273102 | AML, MDS | Completed |
| ORY-1001 |  | Phase I | EudraCT 2013-002447-29 | Relapsed or refractory AL | Completed |
| | | Phase I | NCT02913443 | Relapsed, extensive-stage disease SCLC | Completed |
| | | Preclinical | EudraCT 2018-000469-35 | Relapsed, extensive-stage disease SCLC | Ongoing |
| | | Phase II | EudraCT 2018-000482-36 | AML | Ongoing |
| GSK2879552 |  | Phase I/II | NCT02929498 | High risk MDS | Terminated |
| | | Phase I | NCT02177812 | AML | Terminated |
| | | Phase I | NCT02034123 | Relapsed/refractory SCLC | Terminated |
| | | Phase I | NCT03132324 | Sickle cell disease | Terminated |
| INCB059872 |  | Phase I/II | NCT02712905, EudraCT 2017-001710-28 | Advanced malignancies | Terminated |
| | | Phase Ib | NCT03514407, EudraCT 2018-000062-11 | Relapsed or refractory ES | Terminated |
| | | Phase I/II | NCT02959437 | Advanced solid tumors | Completed |
| IMG-7289 |  | Phase I | NCT02842827 | AML, MDS | Completed |
| | | Phase IIb | NCT03136185 | Myelofibrosis | Recruiting |
| | | Phase II | NCT04081220 | ET | Recruiting |
| | | Phase IIb | NCT04254978 | ET | Recruiting |
| | | Phase II | NCT04262141 | PV | Recruiting |
| | | Phase II | NCT03867253 | Mild to moderate AD | Active, not recruiting |
| ORY-2001 |  | Phase IIa | EudraCT 2018-002140-88 | AD, LBD, ADHD, BPD, ASD | Completed |
| | | Phase IIa | EudraCT 2019-001436-54 | Aggression in AD | Ongoing |
| | | Phase IIa | EudraCT 2017-002838-23 | RRMS, SPMS | Ongoing |
| | | Phase II | EudraCT 2020-001618-39 | ARDS in patients with COVID-19 | Ongoing |
| CC-90011 |  | Phase I | NCT02875223, EudraCT 2015-005243-13 | Relapsed and/or refractory solid tumors and non-Hodgkin's lymphomas | Recruiting |
| | | Phase II | NCT04350463 | Advanced cancers | Recruiting |
| | | Phase I/II | NCT03850067 | First line, extensive stage small cell lung cancer | Recruiting |
| SP-2509 |  | Phase I | NCT03600649 | Relapsed or refractory ES | Ongoing |
| | | Phase I | NCT03895684 | Advanced solid tumors | Ongoing |

electron from the nitrogen atom of TCP to form a cation radical, followed by cleavage reactions of the cyclopropyl ring to yield the C (**4a**) adduct **A** and five-membered ring adduct **B**.[33] The covalent binding of two isomers of chiral TCPs with FAD gives N (**5**) adducts **C** and **D**.[34,35] As shown in Figure 1C, the covalent TCP-FAD adduct is located in a hydrophobic pocket surrounded by V333, H564, T335, Y762, A899, and T810 residues. The benzene ring of the adduct has weak van der Waals forces with the methyl groups of T810 and T335 residues but has no additional contacts with the surrounding hydrophobic residues. This binding model indicates that the incorporation of hydrophobic substituents into the benzene ring may form more interactions with these hydrophobic residues, thus inhibiting LSD1 with higher potency.[33]

## 3. TCP-BASED LSD1 INHIBITORS

As LSD1 shares a similar catalytic structural domain and mechanism with other monoamine oxidases (MAOs), three known anti-MAO agents, including TCP, pargyline, and phenelzine, were evaluated for their LSD1 inhibitory activity. Among them, TCP was determined to be the most potent one with moderate LSD1 inhibitory activity ($K_i$ = 243 $\mu$M) but poor selectivity.[16,36,37] Currently, TCP is undergoing three clinical trials in combination with all-trans-retinoic acid


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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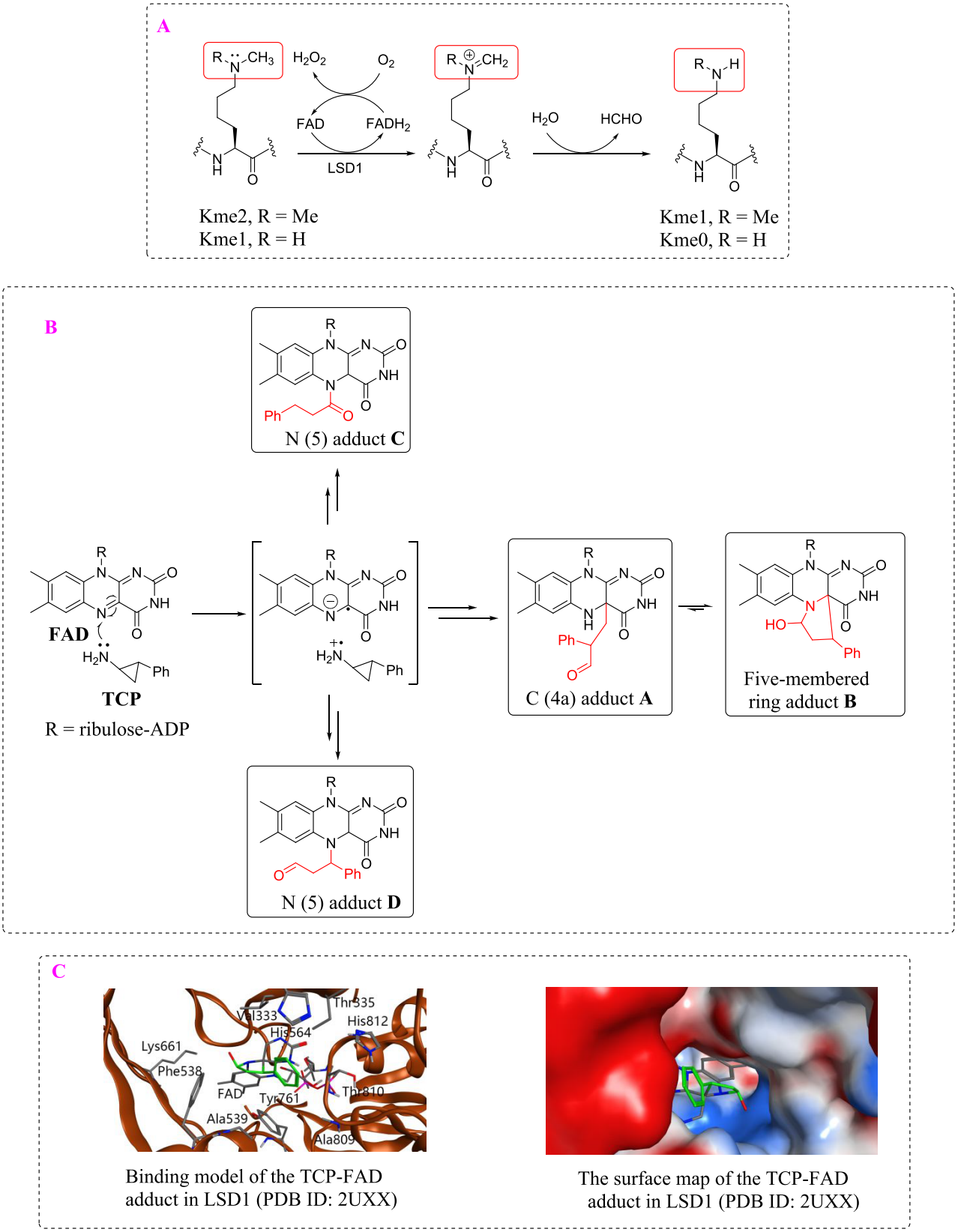


**Figure 1.** Structural basis for designing novel TCP-based LSD1 inhibitors. (A) Catalytic mechanism of LSD1. (B) Mechanism of LSD1 inhibition by forming the covalent TCP−FAD adducts. (C) Cocrystal structure of TCP−FAD adduct in LSD1.

(ATRA) to treat AML and myelodysplastic syndromes (MDS). In 2014, phase I/II trials were launched to assess the feasibility, safety, and biological activity of TCP/ATRA combination therapy for treating relapsed or refractory AML patients and AML patients who cannot tolerate intensive therapy (NCT02261779, EudraCT 2012-002154-23). Eight-



https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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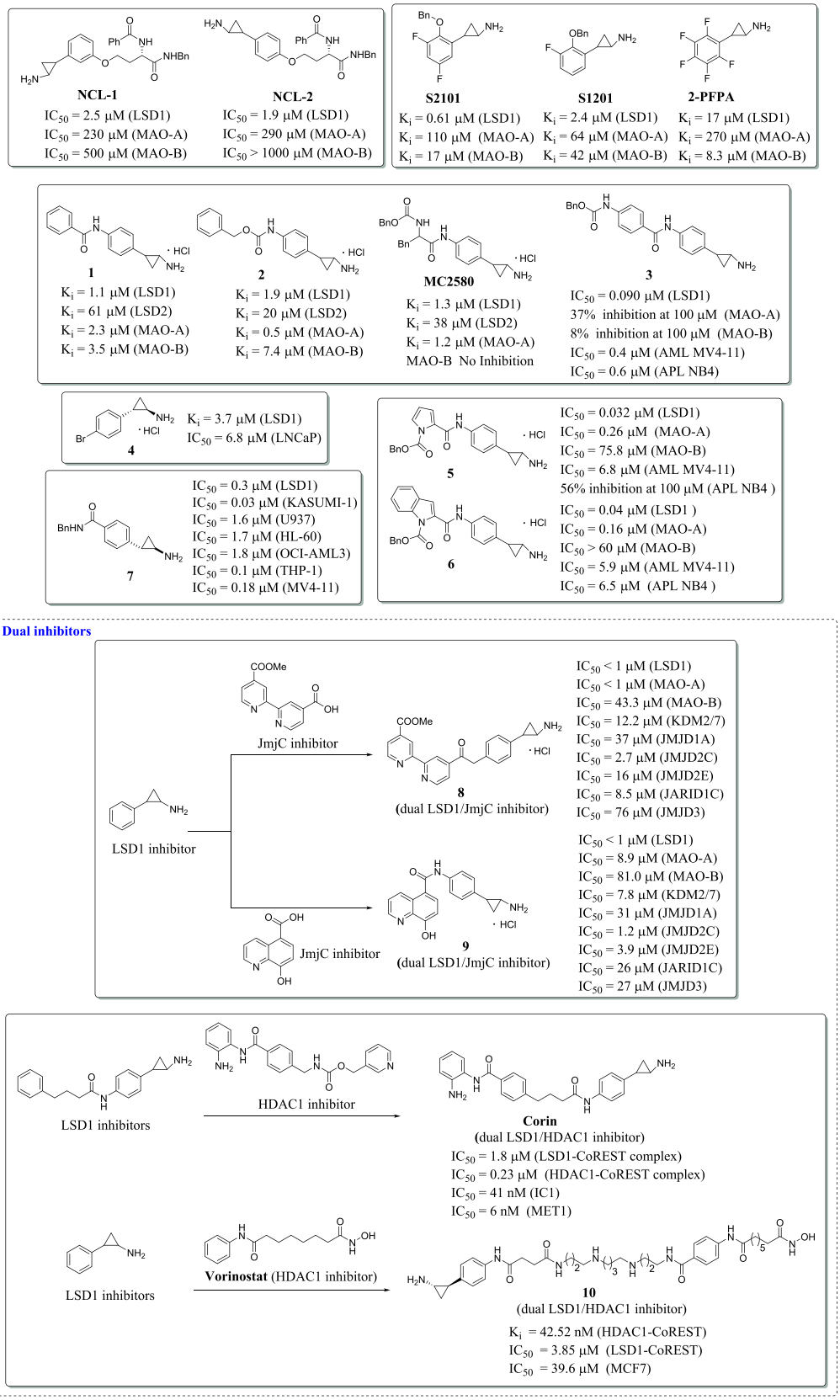


**Figure 2.** Compounds substituted on the phenyl of TCP.

een patients were treated with this combined treatment, and the overall response rate was 20%. Patients who did not reach clinical remission were observed for myeloid differentiation. Median overall survival (OS) was only 3.3 months. Increased levels of H3K4me1 and H3k4me2 were observed in AML blasts and white blood cells from several treated patients.[38] Another phase I trial was designed to explore the safety and tolerability of TCP/ATRA combination therapy for treating


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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AML and MDS patients (NCT02273102). In 2016, the phase I/II study of sensitization of non-M3 AML blast cells to TCP/ATRA combination was initiated to determine the maximum-tolerated dose of TCP combined with fixed-dose ATRA and with fixed-dose Ara-C, to obtain the recommended phase II dose for AML or MDS patients and to evaluate the efficacy (NCT02717884). In 2020, Zhang et al. revealed that cotreatment with TCP and GSK-J1 (JMJD3 inhibitor) had synergistic therapeutic effects against head and neck squamous cell carcinoma *in vitro* and *in vivo*.[39] The cocrystal structure of the LSD1−CoREST complex revealed that the activity pocket of LSD1 is larger and more hydrophilic than those of MAO-A and -B, which provides an important structural basis for developing novel potent and selective LSD1 inhibitors that fully engage neighboring residues.[33]

In this Perspective, we will summarize the chemical structures, bioactivities, and structure−activity relationship (SAR) of TCP-based LSD1 inhibitors reported in recent years and discuss their potential as therapeutic drugs. They are divided into five categories according to their molecular structure and substitution position, including compounds substituted on the phenyl of TCP, compounds substituted on the amino of TCP, miscellaneous substituted TCP-based compounds, compounds substituted on the cyclopropyl core of TCP, and conformationally constrained TCP-based compounds.

### 3.1. Compounds Substituted on the Phenyl of TCP.

TCP is a weak and nonspecific LSD1 inhibitor; making substitutions on the phenyl ring to enhance its potency and specificity has been pursued in recent years.[23] In 2008, Gooden et al. designed the first series of TCP derivatives with substituents attached to the benzene ring and examined the inhibition kinetics for some representative compounds against LSD1.[40] Some derivatives (*para*-$CF_3$, -Br, -Me) displayed higher LSD1 inhibitory activity than TCP but poor LSD1 selectivity against MAO-A and -B. Based on the cocrystal structures of TCP/*N*-propargyl lysine peptide−FAD adducts,[41] NCL-1 and NCL-2 were designed by Miyata et al. in 2009, featuring the side chain of an amino acid at the *meta*- and *para*-positions of the TCP benzene ring, respectively (Figure 2).[42] The introduced benzylamino and benzoyl groups could be recognized by several hydrophobic amino acid residues around the LSD1 active site, and the resulting compounds showed enhanced cell permeability. Both NCL-1 and NCL-2 exhibited good selectively for LSD1 over MAO-A and -B. Furthermore, crystal structure analysis showed that the active pockets of MAO-A and -B[43,44] do not have sufficient space to accommodate bulky substituents on the TCP benzene ring, underscoring the importance of the amino acid moiety for LSD1 inhibitory activity and selectivity. Next, NCL-1 and NCL-2 were identified as cell-active LSD1 inhibitors against many kinds of cancer cell lines ($GI_{50}$ = 6−67 $\mu$M). Following studies confirmed that NCL-1 exhibited good anticancer activities in several solid tumors, including breast cancer,[45] prostate cancer,[46,47] and glioma.[48,49] Also, high-dose NCL-1 caused dysfunctional spermatogenesis and induced caspase-dependent apoptosis.[50]

In 2010, Mimasu et al. designed a new series of LSD1 inhibitors based on TCP-inhibited structural superposition of LSD1 and MAO-B.[51] Among them, S2101 was the most active ($K_i$ = 0.61 $\mu$M) and exhibited stronger LSD1 inhibitory potency than TCP due to the *ortho*-substituent of the TCP benzene ring and two fluorines at *meta*-positions (Figure 2). In addition, S2101 showed excellent selectivity for LSD1 over MAO-A and -B. The inhibition modes of compounds S2101 and 2-PFPA were also identified by crystal structure analysis, which revealed that a benzene ring at the *ortho*-position of TCP improved the inhibitor−FAD adduct stability through extra contacts with the surrounding LSD1 residues. In human HEK293T cells, S2101 exhibited about 50-fold stronger LSD1 inhibitory activity than TCP.

In the same year, Binda et al. designed the first series of 4-amidophenylcyclopropylamine analogues by introducing some different large hydrophobic and hydrophilic groups at the *para*-position of the TCP benzene ring, such as compounds **1**, **2**, and MC2580 (Figure 2).[35] These derivatives were used to obtain more information about the differences in the binding sites of LSD1, lysine-specific demethylase 2 (LSD2/KDM2A), and MAO-A and -B to further enhance their LSD1 inhibitory selectivity. Among them, MC2580 exhibited the best LSD1 inhibitory potency and selectivity (LSD1 $K_i$ = 1.3 $\mu$M, LSD2 $K_i$ = 38 $\mu$M, MAO-A $K_i$ = 1.2 $\mu$M, no inhibition for MAO-B). The striking potency difference observed in MAO-A and -B is due to the larger and more flexible MAO-A active cavity to accommodate these bulky molecules, which is not present in MAO-B.[35,44] As APL is associated with the aberrant activities of several chromatin modifications, treating APL-derived NB4 cells with MC2580 for 6 h but not more than 12 h caused a selective and concentration-dependent increase in H3K4me2, indicating the irreversible mechanism of action of MC2580. In addition, MC2580 showed an obvious synergistic effect to inhibit cell growth and induce the differentiation of APL cells when combined with retinoic acid.[16] In 2020, the same group designed new TCP analogues carrying double-substituted benzamide residues at the *para*-position of their benzene rings. Among them, the most active compound, **3** (Figure 2), exhibited potent LSD1 inhibitory activity ($IC_{50}$ = 90 nM) and high selectivity against MAO-A and -B. More importantly, compound **3** exhibited sub-micromolar cell growth inhibition against AML cell line MV4-11 ($IC_{50}$ = 0.4 $\mu$M) and APL cell line NB4 ($IC_{50}$ = 0.6 $\mu$M).[52]

In 2011, the enantioselective synthesis of TCP analogues designed as LSD1 inhibitors was first reported by Benelkebir et al.[53] The biological activity results indicated that the two enantiomers of TCP and its racemic mixture exhibited similar LSD1 inhibitory activity. Nevertheless, it was found that the activity of (1*R*,2*S*)-**4** (*para*-bromo-tranylcypromine) was over 1000-fold higher than that of TCP in the prostate cancer cell line LNCaP. The enhanced cell-based activity of (1*R*,2*S*)-**4** may be due to its improved cell permeability and lipophilicity compared with TCP (Figure 2).

In 2015, *Z*-pyrole- and *Z*-indole-TCP derivatives that can interact more effectively with the binding pocket to improve LSD1 inhibitory activity and selectivity were developed by Rodriguez et al. (Figure 2).[54] The substituted pyrroles or indoles were then used to replace the chiral amino acid portion of MC2580 to develop novel derivatives with simplified structures. Among these two new series, the most effective compounds, **5** and **6**, displayed high LSD1 inhibitory potency ($IC_{50}$ = 32 nM and 40 nM, respectively). At the cellular level, they can inhibit LSD1 in NB4 cell lines, increase the expression of growth factor independent 1B (GFI-1B) and integrin alpha M (ITGAM), and block the growth of MV4-11 (AML cell line) and NB4 (APL cell line).

In 2020, Borrello et al. developed a series of novel TCP analogues with a carboxamide group at the *para*-position of the


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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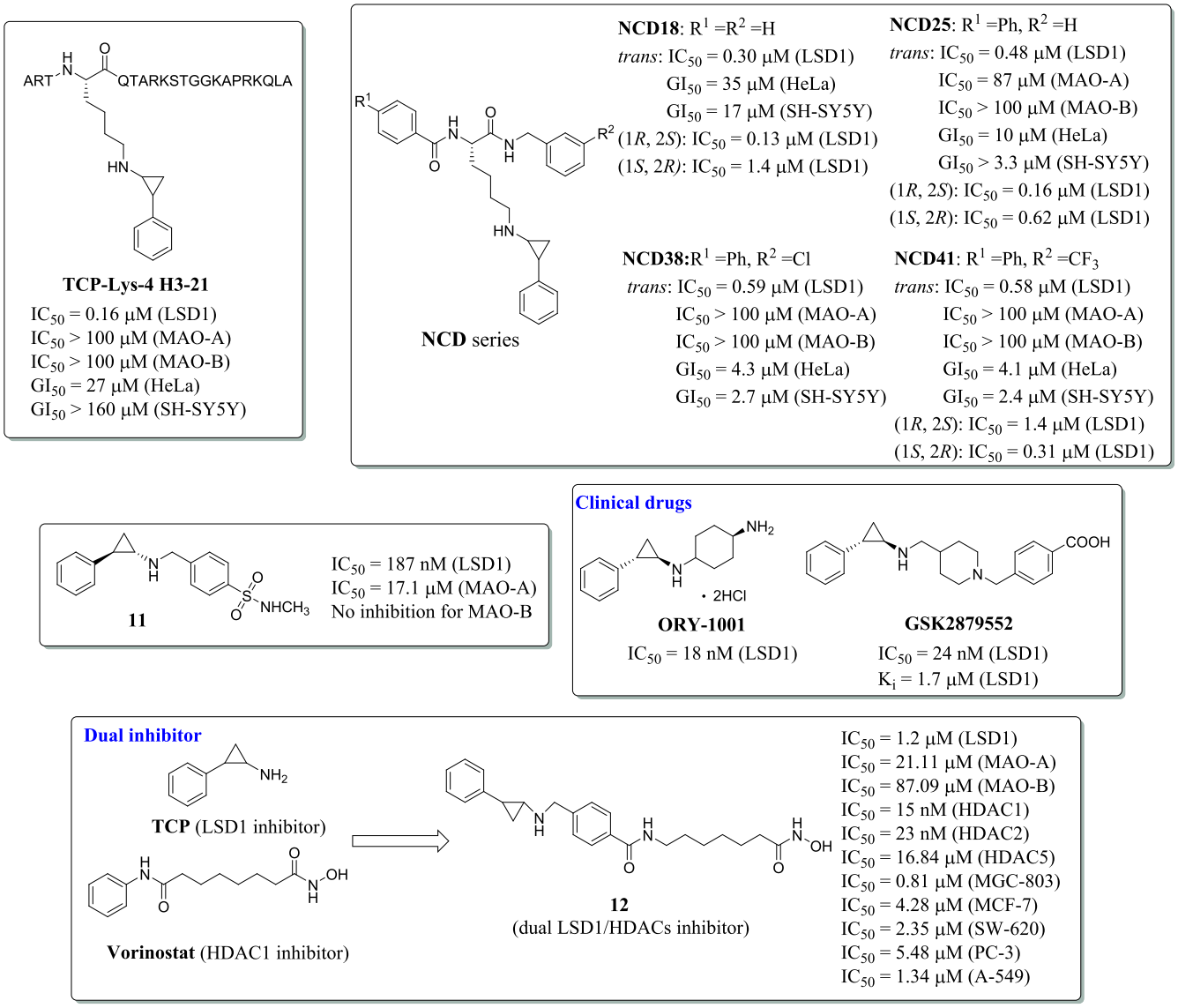


**Figure 3.** Compounds substituted on the amino of TCP.

benzene ring (Figure 2).[55] These newly designed compounds were reverse amide isosteres of previously reported LSD1 inhibitors to avoid the toxicity caused by hydrolysis of the aniline amide. These compounds (such as the most effective compound **7**, which had sub-micromolar LSD1 inhibitory activity) exerted antiproliferative effects against a panel of AML cells. The participation of the LSD1 target in cells was proven by the effect on H3K4me2 protein expression and CD86, CD11b, and CD14 levels.

LSD1 and Jumonji C (JmjC) domain-containing family members histone lysine demethylase 4A/C (KDM4A/C) are coexpressed and colocalized with the androgen receptor (AR) in prostate cancer. By combination of TCP with two JmjC inhibitors (4-carboxy-4′-carbomethoxy-2,2′-bipyridine and 5-carboxy-8-hydroxyquinoline), dual histone demethylase inhibitors (such as hybrids **8** and **9**) simultaneously inhibited LSD1 and JmjC KDMs (Figure 2).[56] In addition to promoting the methylation of H3K4 and H3K9, they also induced growth arrest and extensive apoptosis in prostate cancer cell line LNCaP and colon cancer cell line HCT 116, while little and no apoptosis were induced by compounds **8** and **9** in mesenchymal progenitor cells, demonstrating selective inhibition of cancer.

Histone deacetylases (HDACs) are also effective targets for anticancer therapy, and the HDAC1/2−LSD1−CoREST complex is usually associated with silencing of tumor suppressor genes.[57,58] Hence, designing a dual-targeting agent is a good strategy for medicinal chemists to develop new candidate drugs. In 2018, Kalin et al.[59] reported a synthetic hybrid compound, Corin, that was derived from TCP and entinostat (an HDAC inhibitor) (Figure 2). Corin exhibited dual inhibitory effect on LSD1 and HDAC, which was consistent with the mechanism that the active sites of HDAC1 and LSD1 were dually engaged by Corin's warheads. Compared to its parent monofunctional inhibitors, Corin showed more potent antiproliferation properties in melanoma and cutaneous squamous cell carcinoma cell lines.[59] Moreover, Anastas et al. revealed that co-inhibition of HDACs and LSD1 with Corin potently decreases diffuse intrinsic pontine glioma (DIPG) cell growth *in vitro* and *in vivo* by promoting cell death and differentiation while suppressing cell cycle, which might provide a promising strategy for treating DIPG by simulta-


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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neously inhibiting LSD1 and HDACs.[60] In the same year, Milelli et al.[61] designed a series of polyamine-based dual HDAC/LSD1 inhibitors by coupling vorinostat and TCP. Considering that polyamines can form additional interactions with negatively charged amino acids, different polyamines were used as linkers in this study, and compound **10** emerged as the most potent one, exhibiting excellent inhibitory activity toward HDAC1 ($K_i$ = 42.52 nM) and LSD1 ($IC_{50}$ = 3.85 $\mu$M). In addition, compound **10** displayed stronger antiproliferation activity than vorinostat in breast cancer cell line MCF7 ($IC_{50}$ = 39.6 $\mu$M).[61]

**3.2. Compounds Substituted on the Amino of TCP.** Most LSD1 inhibitors currently under clinical investigation are N-alkylated TCP derivatives. In 2013, using a ligand-based approach, a series of cyclic and short linear peptides were discovered as potent LSD1 inhibitors.[62,63] In the same year, Ogasawara et al. designed a series of LSD1 inhibitors coupling TCP with a histone 3 mimetic peptide (Figure 3).[64] Among them, TCP-Lys-4 H3-21 exhibited high LSD1 potency ($IC_{50}$ = 0.16 $\mu$M) and high selectivity against MAO-A and -B ($IC_{50}$ > 100 $\mu$M). Nevertheless, TCP-Lys-4 H3-21 was found to be inactive in cancer cells possibly due to poor cell membrane permeability. Mechanically, peptide scaffolds served as carriers to deliver TCP into the LSD1 active pocket, followed by forming the TCP−FAD adduct to inhibit LSD1. The SAR studies indicated that the peptide chain length was very important for LSD1 inhibitory potency.[65] This strategy was also employed to develop a series of small-molecule lysine−TCP conjugates (NCD series compounds) with potent antiproliferative effects against cervical cancer cell line HeLa and neuroblastoma cell line SH-SY5Y.[64] In 2014, the same group investigated the LSD1 inhibitory activities of optically pure isomers of lysine−TCP hybrids NCD18, NCD25, and NCD41.[66] It was found that (1*R*,2*S*)-NCD18 and (1*R*,2*S*)-NCD25 were 11-fold and 4-fold more potent than their corresponding enantiomers, respectively. Additionally, the LSD1 activity of (1*S*,2*R*)-NCD41 was about 4 times higher than that of its enantiomer. Molecular docking study suggested that these compounds interacted with hydrophobic amino acid residues and formed hydrogen bonds within the LSD1 catalytic cavity. In addition, the stereochemistry of TCP could also affect the inhibitory activity of LSD1. By regulating apoptosis and suppressing SRY (sex-determining region Y)-box 2 (SOX2) and Oct4, NCD38 and previously mentioned NCL-1 can effectively inhibit the growth of germ cell tumors *in vitro* and *in vivo* without adverse reactions, indicating that NCD38 and NCL-1 have the potential to be used as therapeutic agents for germ cell tumors.[67]

In 2018, Schulz-Fincke et al. designed a series of novel *N*-alkylated TCP derivatives resulting in some highly potent LSD1 inhibitors (Figure 3).[68] Among them, *N*-methyl sulfonamide **11** is the most potent LSD1 inhibitor ($IC_{50}$ = 187 nM) with high selectivity against MAO-A and -B. In this study, the introduction of a polar, nonbasic functional group can not only maintain the inhibitory activity of LSD1 but also improve the selectivity against MAO-A and -B and inhibit colony formation of leukemia cells effectively. Docking studies showed that the wide binding pocket of LSD1 can accommodate the selective binding of lipophilic and polar groups, while in MAO-A, the binding sites are narrower and most of them are lipophilic. These findings well explained the high selectivity of inhibitors with polar substituents.

ORY-1001 (iadademstat; Table 1 and Figure 3), the most potent and selective *N*-substituted TCP-based LSD1 inhibitor to date, was developed by Oryzon Genomics, and it is now undergoing clinical trials to treat AML and solid tumors.[26] In 2013, a phase I clinical study to explore the pharmacokinetics (PK) and safety of ORY-1001 for treating relapsed or refractory acute leukemia (AL) showed that the recommended dose of ORY-1001 was well tolerated and promoted blast differentiation (EudraCT 2013-002447-29). In 2016, another phase I clinical study was initiated to evaluate the safety and tolerability of orally administered ORY-1001 for the treatment of relapsed, extensive-stage disease SCLC (NCT02913443). Combined with platinum-etoposide chemotherapy, preliminary studies to investigate the safety, tolerability, recommended dose, and efficacy of ORY-1001 against relapsed and extensive-stage disease SCLC are currently ongoing (EudraCT 2018-000469-35). Preliminary studies on the safety, tolerability, recommended dose, and efficacy of ORY-1001/azacitidine combination therapy in the first-line treatment of older AML patients are also in progress (EudraCT 2018-000482-36). Very recently, Oryzon Genomics presented new phase II clinical data on ORY-1001 in combination with azacitidine in elderly AML patients at the Congress of European Hematology Association.[69] In biochemical level, ORY-1001 displayed potent LSD1 inhibitory activity ($IC_{50}$ = 18 nM) and high selectivity (>1000-fold higher than LSD2 and MAO-A and -B).[26,70] In THP-1 cells, ORY-1001 induced H3K4me2 accumulation in a dose- and time-dependent manner and induced differentiation by inducing CD11b.[26] Moreover, it can induce THP-1 cell apoptosis and inhibit MV-4-11 cell proliferation and colony formation. Further *in vivo* study also confirmed that ORY-1001 can significantly inhibit the growth of tumors in rodent MV-4-11 xenografts. As a drug in clinical trial, ORY-1001 was found to be stable in hepatocytes with no inhibitory effect on cytochrome P450 enzymes (CYP) and human ether-a-go-go related gene and exhibited high activity, oral bioavailability, and *in vivo* target exposure. When ORY-1001 was applied in combination with standard-of-care drugs (such as ATRA, Ara-C/cytarabine, and quizartinib) and epigenetic inhibitors (such as DOT1L inhibitors EPZ-5676 and SGC-0946, DNMT1 inhibitors decitabine and azacitidine, HDAC inhibitor SAHA, and BCL2 inhibitor ABT-737) in MV-4-11, MOLM13, and MOLT4 cell lines; these combinations significantly suppressed tumor growth in an AML-xenograft model and prolonged survival in a T-cell AL patient derived xenograft (PDX) model.[26,71] A recent report also indicated that ORY-1001/OTX015 (a BET protein inhibitor) combination treatment exhibited synergistic lethality effects on blast progenitor cells of AML.[72] ORY-1001 also exhibited good inhibitory effect on the xenograft growth of responsive SCLC cell line NCI-H510A, but it was not very effective for the SCLC cell line NCI-H526.[73] In 2020, Cuyàs reported that ORY-1001 inhibited breast cancer stem cell (CSC)-driven mammosphere formation, blocked the LSD1-targeted SOX2 enhancer activation, and significantly and selectively reduced the formation of mammospheres by CSC-like cells from a multidrug-resistant luminal-B breast cancer PDX.[74]

As one of the first two inhibitors in clinical trials, GSK2879552 (Table 1 and Figure 3) is an orally active, potent, and selective *N*-substituted TCP-based LSD1 inactivator identified from 2.5 million compounds. With the aid of the cocrystal structure of the GSK2879552−FAD adduct


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
J. Med. Chem. 2020, 63, 14197−14215

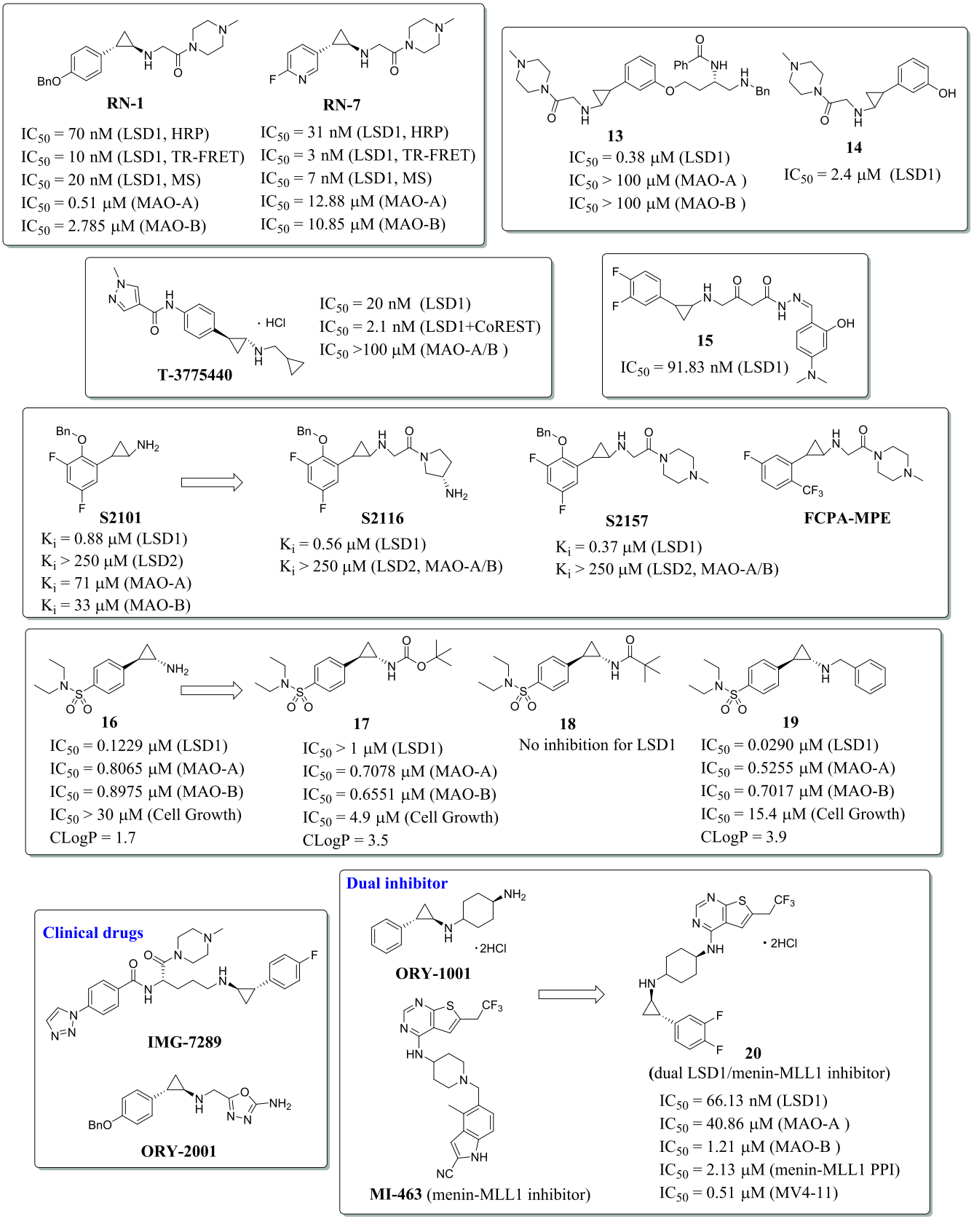


**Figure 4.** Miscellaneous substituted TCP-based compounds.

(PDB ID 6NQU), GSK2879552 was characterized as a mechanism-based inhibitor depending on the LSD1 catalytic activity.[27] Moreover, anticancer activity evaluation using a panel of 165 cell lines suggested that GSK2879552 is narrowly active in SCLC and AML, and DNA hypomethylation may act as a biomarker to predict the response to GSK2879552 in


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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SCLC. GSK2879552 has entered phase I/II clinical trials for treating high-risk MDS alone or in combination with azacytidine (NCT02929498), and two phase I clinical studies to treat AML (NCT02177812) and relapsed/refractory SCLC (NCT02034123), but all these clinical trials have been terminated due to the high incidence of some serious adverse events.

INCB059872 (Table 1 and Figure 3) is a new potent TCP-based small-molecule LSD1 inhibitor reported by Incyte Corporation in 2016.[29,30] In preclinical models of human SCLC, INCB059872 can effectively inhibit SCLC cell proliferation with $EC_{50}$ values ranging from 47 to 377 nM.[29] INCB059872 is presently undergoing four clinical trials for anticancer treatment. A phase I clinical study assessing the safety, pharmacokinetics, and efficacy of INCB059872 in patients with sickle cell disease was discontinued in 2019 due to a business decision not to pursue INCB059782 in sickle cell disease indication (NCT03132324). A phase I/II dose-escalation/expansion and safety study of INCB059872 in patients with advanced malignancies (NCT02712905, EudraCT 2017-001710-28) and a phase I clinical trial concerning the evaluation of the safety and preliminary efficacy of INCB059872 in relapsed or refractory Ewing sarcoma (ES) (NCT03514407, EudraCT 2018-000062-11) were also terminated due to business decision. A phase I/II study to explore the safety and tolerability of INCB059872 in combination with pembrolizumab and epacadostat for treating advanced solid tumors has been completed recently (NCT02959437). Further, a "basket" study of new therapies for MDS or myeloproliferative disorders is scheduled to conduct clinical I/II trials in 2020 (NCT04061421).

In 2017, Duan et al. designed a series of LSD1/HDACs dual inhibitors combined with *N*-substituted TCP derivatives and pharmacophore of HDAC inhibitors (Figure 3).[75] Among them, compound **12** showed the highest inhibitory activity against LSD1 ($IC_{50}$ = 1.20 $\mu$M), HDAC1 ($IC_{50}$ = 15 nM), and HDAC2 ($IC_{50}$ = 23 nM). The antiproliferative activities of compound **12** ($IC_{50}$ = 0.81−5.48 $\mu$M) against four cancer cell lines (MGC-803, MCF-7, SW-620, and A-549) are stronger than that of SAHA. Molecular docking experiments indicated that compound **12** fits well with the binding pockets in LSD1 and HDAC2 active sites, which provided potential prospects to develop more effective dual LSD1/HDACs inhibitors for cancer therapy.

**3.3. Miscellaneous Substituted TCP-Based Compounds.** In 2011, Neelamegam et al. reported new TCP derivatives by introducing different substituents at the benzene ring and amino group as LSD1 inhibitors for the treatment of brain disorders (Figure 4).[76] Among them, RN-1 and RN-7 exhibited the highest LSD1 inhibitory potency ($IC_{50}$ = 10 nM and 3 nM, respectively) and moderate selectivity against MAO-A and -B. A novel object recognition test was used to evaluate the influence of RN-1 on long-term memory. Mice systemically treated with RN-1 developed significant long-term memory impairment with short-term memory intact. In a subsequent study, it was reported that RN-1 is a promising $\gamma$-globulin inducer to treat sickle cell disease.[77,78]

NCL-1 was the first cell-active LSD1 inhibitor designed by Ahmed Khan; in 2015, the same group further developed a series of *N*-alkylated NCL-1 analogues with higher LSD1 inhibitory potency and selectivity (Figure 4).[79] Among them, compound **13** displayed LSD1 inhibitory activity about 6 times stronger than that of NCL-1 and compound **14**. Moreover, compound **13** exhibited moderate cellular activity with $GI_{50}$ of 42 $\mu$M in SH-SY5Y cells, but highly polar compound **14** was inactive in SH-SY5Y cells due to the poor cell membrane permeability.

In 2017, Takeda Pharmaceuticals identified T-3775440 as a novel *N*-alkylated TCP-based LSD1 inhibitor (Figure 4).[80] T-3775440 exhibited high LSD1 inhibitory potency with $IC_{50}$ values of 2.1 nM in the presence of CoREST and 20 nM without CoREST. In addition, T-3775440 showed excellent LSD1 selectivity against MAO-A and -B. At a cellular level, T-3775440 also inhibited the growth of AML and acute megakaryoblastic leukemia (AMKL) cells by inducing cell transdifferentiation. When T-3775440 was applied in combination with NEDD8-activating enzyme (NAE) inhibitor pevonedistat in AML, they exhibited a synergistic effect for AML treatment.[81]

Inspired by the potency of the TCP skeleton and hydrazone moiety, in 2017, our group developed a series of phenylalanyl hydrazone based TCP derivatives as novel small-molecule LSD1 inhibitors (Figure 4).[82] Among these newly synthesized compounds, compound **15** exhibited the strongest LSD1 inhibitory effect ($IC_{50}$ = 91.83 nM). In addition, compound **15** induced the accumulation of CD86 and H3K4me2 and inhibited gastric cancer cell proliferation. Our findings indicated that compound **15** can serve as a promising lead compound to target gastric cancer.

In 2020, Niwa et al. introduced (2-aminopyrrolidine-1-yl) ethanone (APE) and (4-methylpiperazine-1-yl) ethanone (MPE) at the amino group of the previously reported S2101 to yield novel *N*-alkylated TCP-based LSD1 inhibitors (Figure 4).[83] Compared with S2101, newly synthesized compounds S2116 and S2157 showed improved LSD1 inhibitory potency with $K_i$ values of 0.56 $\mu$M and 0.37 $\mu$M, respectively. S2116 and S2157 also showed high LSD1 inhibitory selectivity against MAOs, which may be due to steric restraints. The reported compound FCPA-MPE, bearing a fluoro-trifluoromethylphenyl group at the *para*-position of the benzene ring and MPE on the amino group of TCP, exhibited higher activity and selectivity for LSD1 inhibition than S2101. Crystal structural analysis of the complexes of LSD1 with S2101, S2116, S2157, and FCPA-MPE revealed that the *N*-substituents enhanced the LSD1 inhibitor activity without forming adducts with FAD.[83]

Sulfonamide is one of the most important amide isosteres commonly used in drug development.[84] In 2020, Liang et al.[85] introduced a sulfonamide group at the *para*-position of the TCP benzene ring to develop a novel series of LSD1 inhibitors (such as compound **16**, Figure 4). Its LSD1 inhibitory activity was significantly improved by the introduction of sulfonamide. In addition, compound **17** obtained by introducing a Boc (*tert*-butoxycarbonyl) group at the amine group of compound **16** significantly increased antiproliferation activity against AML cells. Additional intracellular thermal shift and mass spectroscopy showed that the lipophilic Boc group was beneficial to cellular uptake and it can be easily removed under acidic conditions to obtain the real LSD1 inhibitors, which was further proved by the fact that an acidic inert pivaloyl replacing the Boc group (such as compound **18**) substantially reduced the antiproliferative effect on AML cells. Finally, compound **19** with appropriate lipophilicity was developed, which exhibited better LSD1 inhibitory activity and antiproliferative activity in AML cell lines.[85]

IMG-7289, bomedemstat (Table 1 and Figure 4) is an oral small-molecule LSD1 inhibitor developed by Imago Bio-


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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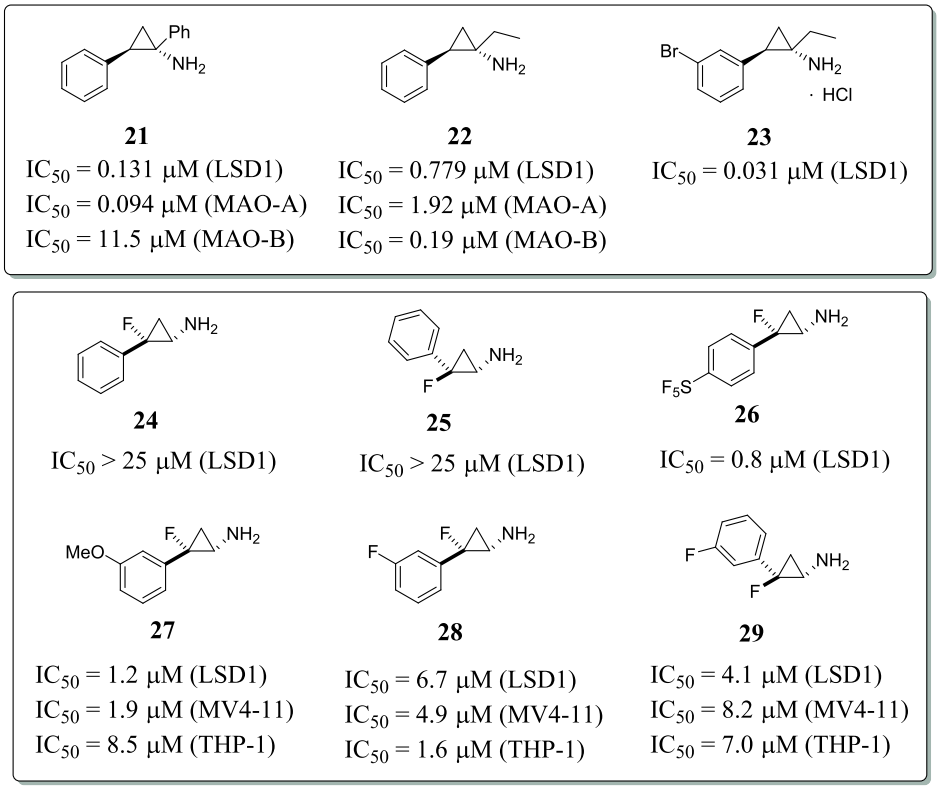


**Figure 5.** Compounds substituted on the cyclopropyl core of TCP.

Sciences. A phase I clinical study of IMG-7289 alone or in combination with ATRA for treating AML and MDS has been completed in late 2018 (NCT02842827), but the clinical results have not yet been published. In addition, IMG-7289 transitioned from a phase I/IIa dose-finding study to a phase IIb trial to evaluate its safety, steady-state pharmacokinetics, and pharmacodynamics for the treatment of myelofibrosis (MF) (NCT03136185). The recently published clinical trial results revealed that IMG-7289 can be well tolerated in heterogeneous patients with advanced MF and limited treatment options, and it can also improve symptom burdens in the majority of patients and modestly reduce spleen sizes in some patients.[86] In 2018, Jutzi et al.[87] reported that IMG-7289 can normalize or improve blood cell counts, reduce spleen sizes, restore normal splenic architecture, decrease mutant allele burden, reduce marrow fibrosis, and improve survival in a $JAK2^{V617F}$ mouse model of myeloproliferative neoplasms (MPNs). Moreover, the combination of IMG-7289 and ruxolitinib (a JAK1/2 inhibitor) showed synergistic effects on normalizing the MPN phenotype in mice, which provides a theoretical basis to investigate combination therapy. These data strongly support its clinical study for myelofibrosis treatment.[87] In 2020, a single-center phase II clinical trial (NCT04081220) and a multicenter phase IIb clinical trial (NCT04254978) of IMG-7289 were carried out to treat essential thrombocythemia (ET). Furthermore, a phase II clinical study was initiated in 2020 to assess the hematologic effects of IMG-7289 therapy in ET and polycythemia vera (PV) patients that failed at least one standard therapy (NCT04262141).

ORY-2001, vafidemstat (Table 1 and Figure 4), reported by Oryzon Genomics, is a novel orally bioavailable and brain penetrable TCP-based dual inhibitor for LSD1 and MAO-B.[88] Preclinical data from animal studies showed that ORY-2001 can restore memory and reduce aggressiveness and social avoidance of senescence-accelerated mouse prone 8 (SAMP-8) with accelerated aging and Alzheimer's disease (AD). In addition, ORY-2001 can also restore behavioral deficits and protein levels of ubiquitin carboxyl-terminal esterase L1 (UCHL1) and Notch1 in SAMP-8 mice[89] and downregulate S100A9 postoperative cognitive impairment and traumatic brain injury (TBI).[90] In a recent phase I clinical trial with healthy volunteers, ORY-2001 was found to be safe and capable of effectively reaching the brain. A phase IIa study to assess the safety and preliminary efficacy of ORY-2001 for treating mild-to-moderate AD was initiated in 2019 (NCT03867253). A Phase IIa "basket" clinical trial to investigate the safety, tolerability, and efficacy of ORY-2001 in aggression in subjects with AD, Lewy body dementia (LBD), borderline personality disorder (BPD), autism spectrum disorder (ASD), and adult attention deficit hyperactivity disorder (ADHD) have been completed in 2019 (EudraCT 2018-002140-88). A parallel clinical study evaluating its safety, tolerability, and efficacy in aggression in a moderate-to-severe AD population was carried out in 2019 (EudraCT 2019-001436-54). In addition, ORY-2001 exhibited a fast, strong, and long-lasting therapeutic effect on multiple sclerosis (MS) in several preclinical models. Now, ORY-2001 is in phase IIa trial for treating relapsing-remitting multiple sclerosis (RR) and secondary progressive (SP) MS (EudraCT 2017-002838-23). Like MERS-CoV, in COVID-19 it has been observed that the cytokines interleukin-6 (IL-6) and interleukin-1$\beta$ (IL-1$\beta$) are central to trigger cytokine storm. In acute preclinical models of inflammation, ORY-2001 was shown to produce a rapid and strong decrease in IL-6, IL-1$\beta$, and other relevant immunomodulatory inflammatory cytokines


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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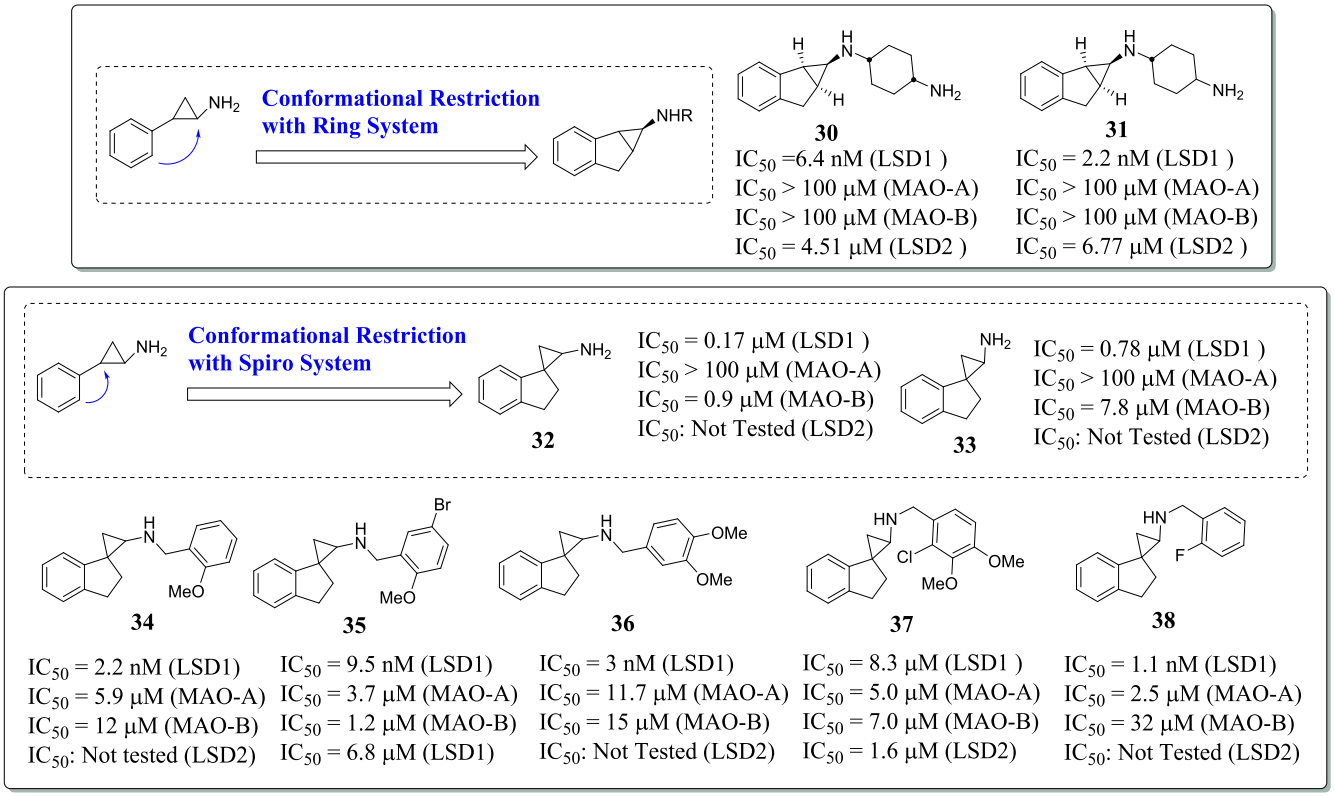


**Figure 6.** Conformationally constrained TCP-based compounds.

including tumor necrosis factor-alpha (TNF-$\alpha$) and interferon-$\gamma$ (IFN-$\gamma$). In 2020, a phase II study was conducted to determine the tolerability and efficacy of ORY-2001 in combination with standard of care treatment to prevent acute respiratory distress syndrome (ARDS) in patients with severe COVID-19 (EudraCT 2020-001618-39).

In 2019, Wang et al. designed several dual LSD1/menin−MLL1 inhibitors that coupled TCP with the 6-trifluoroethylthienopyrimidine skeleton of inhibitors of menin−MLL1 (mixed lineage leukemia 1) interaction (Figure 4).[91] When the newly synthesized inhibitors bind to the menin−MLL1 complex, the TCP moiety is located in the solvent-exposed region of the menin−MLL1 interaction site. Similarly, when they interact with LSD1, the 6-trifluoroethylthiopyrimidine moiety of the molecule remains outside the enzyme. Among these novel chemotypes, compound **20** was the most active LSD1 inhibitor ($IC_{50}$ = 66.13 nM) with excellent selectivity against MAO-A and -B. Moreover, compound **20** showed moderate menin−MLL1 PPI inhibitory activity ($IC_{50}$ = 2.13 $\mu$M) and potent MV4-11 antiproliferative activity ($IC_{50}$ = 0.51 $\mu$M).

**3.4. Compounds Substituted on the Cyclopropyl Core of TCP.** To enhance the understanding of the pharmacological properties of TCP derivatives and to obtain insights into the mechanism-based inhibition caused by the formation of the covalent TCP−FAD adduct, introduction of substituents at the $\alpha$- or $\beta$-position of TCP was carried out to develop more potent LSD1 inhibitors in recent years.[92−94] In 2014, Vianello et al. developed a series of single enantiomers of $\alpha$-substituted TCP derivatives as LSD1 inhibitors.[93] It is worth mentioning that the inhibitory activity of these novel synthesized compounds was strongly dependent on the substitutions on the cyclopropyl ring, and hydrophobic groups (alkyl, phenyl, and benzyl) were also introduced to enhance the LSD1 inhibitory activity. Moreover, introduction of a bulkier group improved the LSD1 selectivity against MAO-A and -B, which can be explained because the catalytic domain of LSD1 is larger than that of MAO-A and -B. Among these newly synthesized compounds, compound **21** (Figure 5) was the most potent one ($IC_{50}$ = 0.131 $\mu$M) with high selectivity against MAO-B, which gave new insight for developing more effective TCP-based LSD1 inhibitors. Compound **21** was also less potent than its opposite enantiomer and racemic counterparts. Further structural and biochemical analysis highlighted that stereoisomers had similar LSD1 inhibitory activity but formed different covalent FAD adducts. Encouraged by these promising results and considered that the mechanism involved the formation of benzyl radical, in the next year, the same group reasoned that small electron-withdrawing or electron-donating groups on the TCP benzene ring may induce significant improvements in the LSD1 inhibitory activity (Figure 5).[92] Among these synthesized 1-substituted TCP analogues, compound **23** exhibited the highest LSD1 inhibitory potency ($IC_{50}$ = 31 nM), 25-fold more active than compound **22**.

In 2017, Borrello et al. designed a series of novel TCP analogues bearing a fluorine at the $\beta$-position of the cyclopropyl ring (Figure 5).[94] Compounds **24** and **25**, adding a fluorine atom to TCP alone, exhibited decreased LSD1 inhibitory activity compared to TCP. However, continuing to introduce additional substitutions at the benzene ring has produced many fluorinated LSD1 inhibitors with higher potency than TCP. Compounds **26−30** with additional substitutions at the *para*- or *meta*-position of the TCP benzene ring were micromolar LSD1 enzyme inhibitors. At the cellular level, selected compounds **27−29** inhibited AML cell (MV4-


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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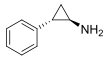
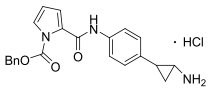
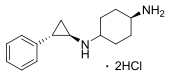
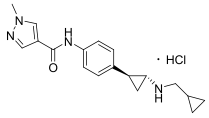
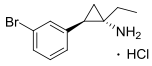
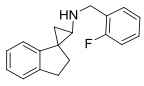
**Table 2. Comparison of Six Classes of TCP-Based LSD1 Inhibitors**

| Classes | The best representative compounds | Potency | Selectivity | Data *in vitro*/*in vivo* |
|---|---|---|---|---|
| TCP |  | $K_i$ = 243 μM | $K_i$ = 102 μM (MAO-A), $K_i$ = 16 μM (MAO-B) | Co-treatment with TCP and GSK-J1 can exhibit synergistic therapeutic effects against head and neck squamous cell carcinoma. |
| Compounds substituted on the phenyl of TCP |  | $IC_{50}$ = 32 nM | $IC_{50}$ = 0.26 μM (MAO-A), $IC_{50}$ = 75.8 μM (MAO-B) | $IC_{50}$ = 6.8 μM (AML MV4-11), 56% inhibition at 100 μM (APL NB4) |
| Compounds substituted on the amino of TCP |  | $IC_{50}$ = 18 nM | >1000 fold selectivity over LSD2 and MAO-A/B | It can induce THP-1 cell apoptosis, inhibit MV-4-11 cell proliferation, colony formation and the growth of tumors in rodent MV-4-11 xenografts. |
| Miscellaneous substituted TCP-based compounds |  | $IC_{50}$ = 20 nM | $IC_{50}$ >100 μM (MAO-A/B) | It can inhibit the growth of AML and AMKL cells by inducing cell transdifferentiation. |
| Compounds substituted on the cyclopropyl core of TCP |  | $IC_{50}$ = 31 nM | NS: not supplied | NS: not supplied |
| Conformationally constrained TCP-based compounds |  | $IC_{50}$ = 1.1 nM | $IC_{50}$ = 2.5 μM (MAO-A), $IC_{50}$ = 32 μM (MAO-B) | NS: not supplied |

11 and THP-1) proliferation and induced H3K4me2 and CD86, which confirmed the LSD1 target engagement.

All these studies mentioned above highlight the potential of modifications at the $\alpha$- or $\beta$-position of the TCP scaffold as a feasible approach to develop novel LSD1 inhibitors with higher potency and selectivity.

**3.5. Conformationally Constrained TCP-Based Compounds.** The development of TCP-based inhibitors is mainly focused on the structural modifications on the benzene ring and the amino groups. Most of these derivatives are racemic mixtures of the *trans*-cyclopropanes; thus the majority of SAR studies were limited to the racemates. Given the expected difference in inhibitory potency of the two enantiomers,[35,95] Ji et al. first presented SAR data of some novel conformationally restricted LSD1 inhibitors based on TCP, including their racemates and enantiomers (Figure 6).[96] Among them, compounds **30** and **31** were identified as the most potent LSD1 inhibitors with excellent selectivity over MAO-A and -B and LSD2. These two compounds were further demonstrated to induce CD86 expression in MV4-11 cells.

Since spiro containing system is not only diverse in orientation but also novel in structure, it is increasingly being utilized in medicinal chemistry. In this case, the spirocycle constraint may have a notable effect on the LSD1 inhibitory activity and selectivity against LSD2 and MAO-A and -B. In 2018, based on the concept of conformational restriction, the same group further designed a series of novel LSD1 inhibitors by introducing the spiro ring at TCP (Figure 6).[97] Compounds **32** and **33**, the simple and direct spirocyclic analog of TCP, exhibited 28- and 129-fold more potent LSD1 inhibitory activity than TCP and little or moderate activity on MAO-B and MAO-A. To further explore the SAR, many different substituted benzyl groups were incorporated into the amino group to develop some novel potent LSD1 inhibitors (compounds **34**−**38**) with excellent selectivity. Further development of more potent and promising spirocyclic TCP-based LSD1 inhibitors deserves particular attention.

## 4. SUMMARY AND PERSPECTIVE

LSD1 specifically removes the methyl group on mono- and dimethylated H3K4 and H3K9,[98] and it is ubiquitously overexpressed in diverse tumors, indicating that LSD1 is an attractive target for cancer therapy. Small-molecule LSD1 inhibitors have been proven to inhibit cancer cell proliferation, invasion, and migration. Since LSD1 was identified in 2004,[2] many types of LSD1 inhibitors with diverse chemical structures, including TCP derivatives, phenelzines, peptides, polyamines, natural products, amidoximes, aminothiazoles triazoles, pyrimidines, and triazole-fused pyridines, have been highly pursued over the past decade, and the most extensively studied LSD1 inhibitors are TCP-based small-molecule compounds.[18,20,22,32,99] In this Perspective, we thoroughly cover the structures, biological activity evaluation, and SAR of TCP-based LSD1 inhibitors in recent years. Currently, six mechanism-based LSD1 inhibitors bearing the TCP skeleton are undergoing clinical trials against cancers alone or in combination with other agents (Table 1), which suggests that TCP is a privileged scaffold for developing new mechanism-based FAD-dependent LSD1 inhibitors. From the aspect of SAR, these substituents and their positions on the TCP


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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scaffold have significant impacts on the LSD1 inhibitory selectivity against MAO-A and -B (Table 2), which helps to further understand the SAR and possible binding model with LSD1 and thereby develop more potent and selective LSD1 inhibitors by specific optimizations on the TCP scaffold. For example, introducing a basic moiety at the phenyl ring may enhance the potency by targeting the negatively charged regions.[100] In addition, according to the structural features of LSD1 and MAO-A and -B, many MAO inhibitors can be used as templates to rationally design new potent LSD1 inhibitors.[19] TCP-based derivatives contain at least two chiral centers, and previous studies have confirmed that the stereochemistry is critical for LSD1 inhibitory activity. Therefore, pharmacological and toxicological characteristics of all isomers should be studied to determine the isomers with higher activity when designing TCP-based LSD1 inhibitors.[100] Additionally, further investigations on the combination of TCP derivatives with other drugs for the treatment of other solid tumors are of significant research interest.[32]

The mechanism of TCP-based LSD1 inhibitors is that TCP binds to FAD in LSD1 covalently and forms adducts in the binding pocket. Similar to TCP, LSD1 inhibitors based on phenelzine or pargyline also exert their inhibitory effects by covalently inactivating FAD. These mechanism-based inhibitors presented several drawbacks including poor selectivity and *in vivo* toxicity due to their irreversible binding with FAD and micromolar affinity with some targets, such as norepinephrine and dopamine transporter.[26,101,102] In addition, although these mechanism-based inhibitors produce enduring effects on the target of interest, they induce lasting off-target effects due to their nonspecific nature. Since the discovery of TCP as an irreversible LSD1 inhibitor, many TCP derivatives have been developed with improved selectivity over MAOs and reduced toxicity, and six TCP-based LAD1 inhibitors are currently being tested in clinical trials (Table 1). Both reversible and irreversible LSD1 inhibitors are attractive for cancer therapy. In comparison with irreversible LSD1 inhibitors, noncovalent reversible LSD1 inhibitors present potential advantages in phenotype and safety, and they are grouped into two categories: non-natural ones and natural products. In recent years, many research groups, including us,[103−110] have designed and synthesized numerous noncovalent reversible LSD1 inhibitors with reduced toxicity, potent preclinical antileukemic effects, and sub-micromolar potencies,[111] including dithiocarbamates, aminothiazole triazoles, pyrimidines, and triazole-fused pyridines. For instance, in 2019, our group identified of a series of triazole-fused pyrimidine derivatives as highly potent LSD1 inhibitors, of which the most potent compound ($IC_{50}$ = 49 nM) inhibited LSD1 reversibly and was highly selective to LSD1 over MAO-A and -B. However, most reversible LSD1 inhibitors are still in the preclinical research stage, except CC-90011 and SP-2509 (seclidemstat) (Table 1). CC-90011, developed by Celgene, a potent, selective, and orally active pyrimidinone-based LSD1 inhibitor, has shown antiproliferative effects against cancer cells *in vitro* and in PDX models. Three clinical studies of CC-90011 alone or combined with other drugs are being conducted for the treatment of relapsed or refractory solid tumors and non-Hodgkin's lymphomas (NCT02875223), advanced cancers (NCT04350463), and SCLC (NCT03850067). SP2509, developed by University of Utah, is a phenylethylidene-benzohydrazide reversible and noncompetitive LSD1 inhibitor with an $IC_{50}$ of 13 nM with no inhibitory activity against MAO-A and -B.[101] Two phase I clinical trials for the treatment of ES (NCT03600649) and solid tumors (NCT03895684) are currently ongoing. Several natural products, baicalin,[112] polymyxins,[113] resveratrol,[107] α-mangostin,[114] *Scutellaria baicalensis* Georgi,[115] curcumin,[116] biochanin A,[117] and protoberberine alkaloids,[118] have been reported to inhibit LSD1; therefore natural products will become rich sources for identifying new reversible LSD1 inhibitors. As FAD is the cofactor of LSD1, compounds sharing similar structure with it can potentially compete for binding to LSD1 by occupying the FAD binding pocket, which represents a promising approach to the development of FAD-competitive reversible LSD1 inhibitors.[103,108] For instance, in 2019, our group reported the FAD (ligand)-based design of fragment-like xanthine derivatives as LSD1 inhibitors, of which the most potent one possessed promising LSD1 inhibitory potency ($IC_{50}$ = 6.45 μM).[108] In previously reported structures, the amino acid residues of the LSD1 active site share similar conformations;[41,119,120] thus the discovery of underexploited binding sites in the substrate-binding region may facilitate the design of new structure-based LSD1 reversible inhibitors. For instance, in 2020, Yang et al. designed macrocyclic peptide reversible LSD1 inhibitors based on the structures of H3 substrate analogues bound to LSD1, of which the most active macrocyclic peptide compound ($K_i$ = 2.3 μM) exhibited >40-fold higher activity than the corresponding linear one, indicating that linear and macrocyclic peptides bind LSD1 differently.[121] Virtual screening against the catalytic pocket of LSD1 has been used to develop novel LSD1 inhibitors, but the high similarity of LSD1 with LSD2 and MAOs makes it difficult to develop highly selective LSD1 inhibitors. Developing molecules targeting other LSD1 demethylase activity domains and allosteric domains may yield potent inhibitors with excellent selectivity.[122]

As many oncogenes or tumor suppressor genes are regulated by multiple factors, some LSD1 inhibitors that can abrogate LSD1 mediated demethylation may not display good anticancer activity. Therefore, combination of LSD1 inhibitors with other drugs may be a promising solution to tackle this problem. LSD1 is also involved in a variety of signaling pathways and performs various functional interactions with other proteins; the combination of LSD1 inhibitors with some other potential targets and the dual inhibitors targeting LSD1 and other disease-associated proteins will lead to more pronounced and synergistic effects, thus providing new opportunities for cancer therapy. For instance, domatinostat (4SC-202), a small-molecule epigenetic modulator that inhibited both HDAC1 and LSD1, exerted a significant antineoplastic effect on urothelial carcinoma cells[123] and had cytotoxic and cytostatic effects on atypical teratoid/rhabdoid tumors, targeting specific cell subpopulations, including those with cancer stem-like features, and can be used as an important potential cancer therapeutic.[124] Furthermore, extensive structural modifications on TCP with other pharmacophores have been carried out to develop more potent inhibitors for clinical evaluation, showing synergistic effect and the promising application of these LSD1 inhibitors in clinical treatment. For instance, in 2017, Ishikawa et al. reported a synergistic effect of T-3775440 (an LSD1 inhibitor) and pevonedistat (an NAE inhibitor), yielding excellent anti-AML effects, which indicated that the combination treatment with LSD1/NAE inhibitors deserves more attention and has great potential for the treatment of AML.[81] A recent paper has shown that LSD1


https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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ablation can stimulate antitumor immunity through induced expression of endogenous retroviruses (ERVs) and enable checkpoint blockade.[125] This study not only identified LSD1 as a promising inducer of antitumor immunity and responsiveness to immunotherapy but also provides a basis for the combination of LSD1 inhibitors and anti-PD-(L)1 for cancer therapy. As mentioned above, the combination therapy could be a feasible therapeutic strategy to improve individual potency of LSD1 inhibitors.

Beyond its most studied demethylase activity, LSD1 demethylase-independent function has also been reported to be involved in the development of cancer in recent years,[126−130] which can explain why it has insufficient catalytic inhibition in several cancers.[131,132] Thus, we anticipate that the development of small-molecule compounds to interfere the demethylase-independent functions of LSD1 will be a promising approach for the prevention and treatment of cancer.

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## ACKNOWLEDGMENTS

This work was supported by National Key Research Program of Proteins (No. 2018YFE0195100 for H.-M.L. and No. 2016YFA0501800), National Natural Science Foundation of China (Project No. 81602961 for Y.-C.Z.; Nos. 81430085 and 81773562 for H.-M.L.), and Science and Technology

Innovation Talents of Henan Provincial Education Department (19IRTSTHN001 for Y.-C.Z.).

## ■ ABBREVIATIONS USED

AD, Alzheimer's disease; ADHD, adult attention deficit hyperactivity disorder; AL, acute leukemia; AMKL, acute megakaryoblastic leukemia; AML, acute myeloid leukemia; APE, (2-aminopyrrolidine-1-yl) ethenone; APL, acute promyelocytic leukemia; AR, androgen receptor; ARDS, acute respiratory distress syndrome; ASD, autism spectrum disorder; ATRA, all-*trans*-retinoic acid; Boc, *tert*-butoxycarbonyl; BPD, borderline personality disorder; CoREST, co-repressor of repressor element-1 silencing transcription factor (REST); CSC, cancer stem cells; CYP, cytochrome P450 enzymes; DIPG, diffuse intrinsic pontine glioma; E2F1, E2F transcription factor 1; ERVs, endogenous retroviruses; ES, Ewing sarcoma; ET, essential thrombocythemia; FAD, flavin adenine dinucleotide; GFI-1B, growth factor independent 1B; HDACs, histone deacetylases; HIF1$\alpha$, hypoxia-inducible factor 1$\alpha$; IFN-$\gamma$, interferon-$\gamma$; IL-1B, interleukin-1B; IL-6, interleukin-6; ITGAM, integrin alpha M; JmjC, Jumonji C; KDM4A/C, histone lysine demethylase 4A/C; LBD, Lewy body dementia; LSD1, histone lysine-specific demethylase 1; LSD2, lysine-specific demethylase 2; MAO, monoamine oxidase; MDS, myelodysplastic syndromes; MF, myelofibrosis; MLL1, mixed lineage leukemia 1; MPE, (4-methylpiperazine-1-yl) ethenone; MPNs, myeloproliferative neoplasms; MS, multiple sclerosis; MYPT1, myosin phosphatase targeting subunit 1; NAE, NEDD8-activating enzyme; OS, overall survival; p53, protein 53; PDX, patient-derived xenograft; PK, pharmacokinetics; PPI, protein−protein interaction; PV, polycythemia vera; SAMP-8, senescence-accelerated mouse prone 8; SAR, structure−activity relationship; SCLC, small cell lung cancer; SOX2, SRY (sex-determining region Y)-box 2; STAT3, signal transducer and activator of transcription 3; TBI, traumatic brain injury; TCP, *trans*-2-phenylcyclopropylamine; TNF-$\alpha$, tumor necrosis factor-alpha; UCHL1, ubiquitin carboxyl-terminal esterase L1

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https://dx.doi.org/10.1021/acs.jmedchem.0c00919
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https://dx.doi.org/10.1021/acs.jmedchem.0c00919
J. Med. Chem. 2020, 63, 14197−14215