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Discovery of new tranylcypromine derivatives as highly potent LSD1 inhibitors

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ABSTRACT

Tranylcypromine (TCP)-based structural modifications lead to the discovery of new LSD1 inhibitors, of which compounds **26b** and **29b** effectively inhibit LSD1 with the IC_{50} values of 17 and 11 nM, respectively and also show good selectivity over MAO-B. Mechanistic studies showed that compound **29b** concentration-dependently induced H3K4me1/2 accumulation in LSD1 overexpressed MGC-803 cells and also inhibited metastasis of MGC-803 cells. Collectively, both compounds could be promising lead compounds for further investigation.

The lysine-specific histone demethylase 1A (known as LSD1 or KDM1A) is the first histone demethylase identified in 2004, which specifically removes methyl groups of histone substrate H3 lysine 4 (H3K4) in flavin adenine dinucleotide (FAD)-dependent manner [1]. LSD1 has fundamental roles in physiological processes, and its dysregulation is closely associated with the occurrence and development of various pathological conditions including cancers, virus infections, neurodegenerative diseases, *etc.* [2–6]. Accumulating evidence have showed that pharmacological inhibition of LSD1 by small molecules or genetic knockdown is an effective strategy in controlling the pathological states [7–9]. These findings suggest that LSD1 is a well-characterized therapeutic epigenetic target [10]. To date, numerous natural and synthetic LSD1 inhibitors have been reported in last decades, showing great promise in cancer therapy [11–14]. Particularly, irreversible LSD1 inhibitors including ORY-1001, tranylcypromine (TCP), ORY-2001, GSK-2879552, INCB059872, IMG-7289, and reversible LSD1 inhibitor CC-90011 have advanced into clinical assessment for the treatment of cancers such as acute myeloid leukemia (AML) and small lung cancer cells (SCLC) (Fig. 1) [15,16]. Some of these clinical candidates have also shown promise for treating myelodysplastic syndromes (MDS), multiple sclerosis (MS), myelofibrosis, and Alzheimer's disease (AD) [17–19]. The success of these LSD1 inhibitors highlight the importance of TCP for designing covalent LSD1 inhibitors. Previous studies have showed that modifications on the TCP scaffold could alter the inhibitory activity against LSD1 and also the selectivity over monoamine oxidases (MAO-A/B) [20–22]. As demonstrated by the clinical candidate ORY-2001, the 1,3,4-oxadiazole ring is linked to the TCP scaffold. We propose that replacement of the 1,3,4-oxadiazole ring in ORY-2001 with other bioisosteres (e.g. the triazole ring) may give new LSD1 inhibitors[23,24]. Herein, we designed the title compounds by introducing the triazole ring to the TCP scaffold in place of the 1,3,4-oxadiazole ring. Additionally, it is well recognized that TCP-based LSD1 inhibitors could form covalent adducts with FAD through the single-electron transfer mechanism[25,26]. Thus, we speculate that the electronic effect of substituents may have certain impact on the inhibitory activity of the title compounds. In this work, we introduced two representative groups, namely trifluoromethyl group (CF_3) and methoxyl group (OCH_3), into the phenyl ring, aiming to examine the effect of substituents with different electronic property on the anti-LSD1 activity. The preliminary structure–activity relationship studies (SARs) of new tranylcypromine derivatives led to the discovery of compounds **26b** and **29b** as highly potent LSD1 inhibitors, both compounds effectively inhibited LSD1 with the IC_{50} values less than 20 nM and could represent promising lead compounds for further development.

The synthetic protocol of 1,4-disubstituted-1,2,3-triazole analogue **23**–**31** was based on the copper-catalyzed azide-alkyne cycloaddition (CuAAC) [27], which required two building blocks, *N*-propargylamines **4** (Scheme 1) and phenyl azides **14**–**22** (Scheme 2). As shown in Scheme 1, *trans*-aminocyclopropanes **1** reacted with Boc_2O in the presence of K_2CO_3, generating the corresponding Boc protected *trans*-aminocyclopropanes **2**, further alkylation with propargyl bromide in the presence of sodium hydride gave the corresponding Boc-protected propargylamines **3** [28]. The obtained compounds **3** underwent the

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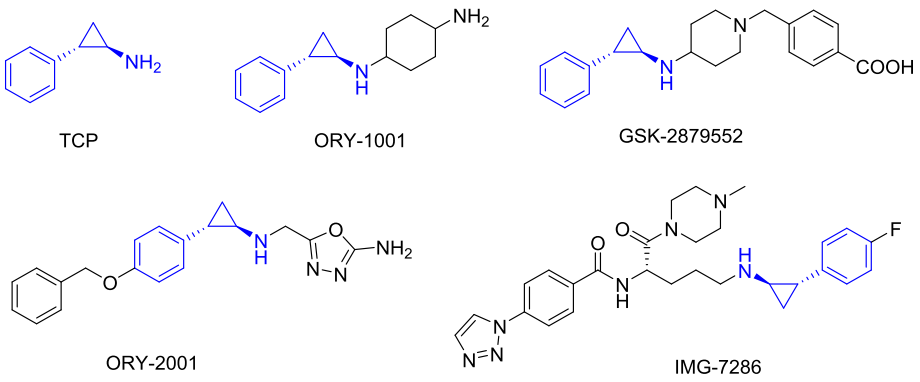
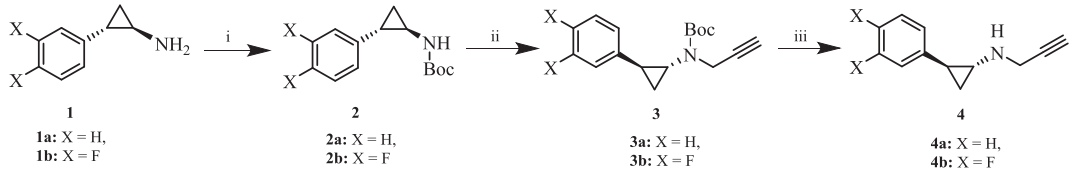
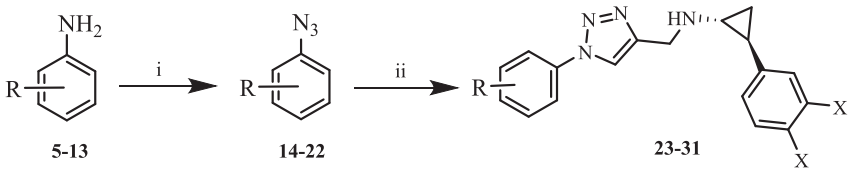


Fig. 1. TCP-based LSD1 inhibitors in clinical trials (TCP is highlighted in blue color).



Scheme 1. Preparation of compounds **4a-b**. Reagents and conditions: i) 1. Boc_2O, DCM, N_2, 0 °C, 0.5 h; 0 °C to RT, 2 h; 2. K_2CO_3, RT, 2 h; ii) 1. NaH, DMF, N_2, 0 °C; 0 °C to RT, 0.5 h; 2. Propargyl bromide, RT, 2 h; 3. Ice, RT, 15 min; iii) HCl, THF, 0 °C to RT, 2 h.



5: R = H,
6: R = 2-OCH_3,
7: R = 3-OCH_3,
8: R = 4-OCH_3,
9: R = 3-CF_3,
10: R = 4-CF_3,
11: R = 2,5-diOCH_3,
12: R = 3,5-diOCH_3,
13: R = 3,5-diCF_3.

14: R = H,
15: R = 2-OCH_3,
16: R = 3-OCH_3,
17: R = 4-OCH_3,
18: R = 3-CF_3,
19: R = 4-CF_3,
20: R = 2,5-diOCH_3,
21: R = 3,5-diOCH_3,
22: R = 3,5-diCF_3.

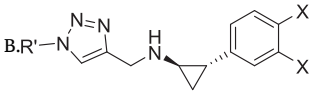
23a: R = H, X = H
23b: R = H, X = F
24a: R = 2-OCH_3, X = H
24b: R = 2-OCH_3, X = F
25a: R = 3-OCH_3, X = H
25b: R = 3-OCH_3, X = F
26a: R = 4-OCH_3, X = H
26b: R = 4-OCH_3, X = F
27a: R = 3-CF_3, X = H
27b: R = 3-CF_3, X = F
28a: R = 4-CF_3, X = H
28b: R = 4-CF_3, X = F
29a: R = 2,5-diOCH_3, X = H
29b: R = 2,5-diOCH_3, X = F
30a: R = 3,5-diOCH_3, X = H
30b: R = 3,5-diOCH_3, X = F
31a: R = 3,5-diCF_3, X = H
31b: R = 3,5-diCF_3, X = F

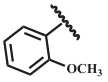
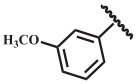
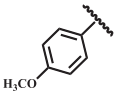
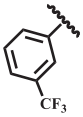
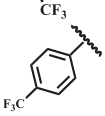
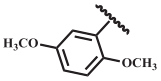
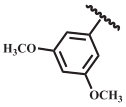
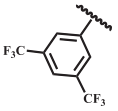
Scheme 2. Preparation of compounds **23**–**31**. Reagents and conditions: i) 1. HCl, $NaNO_2$, H_2O, 0 °C; 2. NaN_3, H_2O, 0 °C to RT, 1 h; ii) *N*-propargylamine, $CuSO_4$, sodium ascorbate, THF/H_2O, RT, 9 h.

deprotection of the *N*-Boc group with dilute hydrochloric acid (6%) to afford the required building blocks *N*-propargylamines **4** (Scheme 2). The synthesis of phenyl azides **14**–**22** started from the appropriate anilines [29]. Treatment of anilines with $NaNO_2$ in acidic aqueous media, followed by azidation in the presence of NaN_3, gave compounds **14**–**22**. The title compounds **23**–**31** were synthesized by the copper-catalyzed click reaction between propargylamines **4** and phenyl azides **14**–**22** (Scheme 2).

With the compounds in hand, we next tested the inhibitory activity of the compounds against LSD1 using the well-known ORY-1001 as the control compound [30]. As shown in Table 1, the compounds showed superior potency against LSD1 with the IC_{50} values as low as 11 nM. Evidently, except for compound **31b**, the remaining compounds bearing the fluorine atoms had better potency than their counterparts without the fluorine atoms, underscoring the importance of the fluorine atom for the potency toward LSD1. Generally, the SARs studies indicated that compounds bearing the electron-donating groups exhibited improved anti-LSD1 inhibitory activity than those substituted with electron-deficient groups. Compared to compound **23b**, compounds **24b**, **25b**, **26b**, **28b**, and **29b** showed comparable or improved potency. Particularly, compounds **26b** and **29b** potently inhibited LSD1 with the IC_{50} values of 17 and 11 nM, respectively. The inhibition cures of the most potent compounds **26b** and **29b** against LSD1 are shown in Fig. 2. For compounds **27b** and **31b** with the –CF_3 group, their inhibitory activity against LSD1 significantly decreased. We also observed that the substituent attached to the 2- or 4-position of the phenyl ring was tolerated, while the one at the 3-position was less tolerated. For example, compounds **24b** and **26b** showed comparable inhibitory activity with compound **23b**, while compound **25b** displayed decreased inhibitory activity with the IC_{50} value lower than that of compound **23b**. The trend was the same for other compounds (**27b** *vs*. **28b**, **29b** *vs*. **30b**). Besides, we also tested the inhibitory activity of the compounds against MAO-A/B to examine the selectivity, and clorgyline and *R*-(-)-deprenyl were used as the reference compounds [31]. As depicted in Table 1, most of the compounds at 10 μM showed excellent inhibitory activity against MAO-A with the inhibitory rates up to 100%, but with relatively lower potency against MAO-B. The results suggest that the compounds may be dual LSD1/MAO-A inhibitors. It has been documented that ORY-2001 is a dual LSD1/MAO-B inhibitor and currently being assessed in clinical trials for the treatment of mild to moderate Alzheimer's disease

Table 1
The inhibitory activity of the compounds against LSD1 and MAO-A/



Compound	R'	X	IC_{50} [nM][a] or inhibition [%][b]		
			LSD1	MAO-A[c]	MAO-B[c]
23a		H	111	102%	76%
23b		F	24	101%	57%
24a		H	120	91%	44%
24b		F	21	90%	51%
25a		H	106	93%	54%
25b		F	42	102%	65%
26a		H	41	99%	55%
26b		F	17	100%	64%
27a		H	110	101%	94%
27b		F	101	90%	43%
28a		H	132	101%	86%
28b		F	26	101%	82%
29a		H	35	97%	58%
29b		F	11	89%	48%
30a		H	110	101%	67%
30b		F	66	93%	42%
31a		H	282	92%	88%
31b		F	664	91%	35%
ORY-1001			0.14	ND[d]	ND[d]
Clorgyline			ND[d]	1.1 nM	ND[d]
R-(-)-deprenyl			ND[d]	ND[d]	70 nM

(a) The IC_{50} values for LSD1 were calculated from 8 data points; (b) Percentage of inhibition at 10 μM; all compounds are single enantiomers. (c) The inhibitory activity of Clorgyline and *R*-(-)-deprenyl against MAO-A/B was examined at 10 different concentrations, and all data are the mean value of two independent determinations. (d) ND means Not Determined.

(ClinicalTrials.gov Identifier: NCT03867253) [32]. The therapeutic potential of such compounds (e.g., **26b** and **29b**) may deserve further investigation.

Encouraged by the high potency of compounds **26b** and **29b** against LSD1 and the selectivity over MAO-B, we also examined their inhibitory activity against LSD1 overexpressed cancer cell lines including PC-3, MCF-7, MGC-803, and SGC-7901. As shown in Table 2, compounds **26b** and **29b** (16 μM) were almost inactive against these cell lines with the inhibitory rates less than 50%, indicating the potential low toxicity. The results are consistent with those previously reported, namely some highly potent and selective LSD1 inhibitors such as ORY-1001 and GSK-2879552 are nontoxic against some cancer cells [30,33].

Considering the favorable potency of compound **29b** against LSD1, additional cellular studies were further conducted to verify its cellular effects in LSD1 overexpressed MGC-803 cells. Then the expressions of two LSD1 substrates, H3K4me1 and H3K4me2, were evaluated in MGC803 cells after exposure to compound **29b** for 72 h. ORY-1001 was used as the positive control. As shown in Fig. 3A, compound **29b** concentration-dependently induced accumulation of H3K4me1 and H3K4me2, supporting that compound **29b** could inhibit the LSD1 activity *in vitro*. Then, the migration ability of MGC-803 cells was further evaluated by the transwell and wound healing assays. As shown in Fig. 3B, compound **29b** suppressed the MGC-803 cell migration in a concentration-dependent manner compared to control group. And further wound healing assay (Fig. 3C) showed that for the untreated group, MGC-803 cells filled almost all the wounded area after scratching the cell monolayer, while compound **29b** concentration-dependently inhibited the wound healing obviously. All the data demonstrated that compound **29b** could block the metastasis of LSD1-overexpressed MGC-803 cells.

In summary, we have performed the structural modifications of TCP, leading to the discovery of compounds **26b** and **29b**, which inhibited LSD1 potently with the IC_{50} values of 17 and 11 nM, respectively and also exhibited good selectivity over MAO-B. The SARs studies revealed the structural features for LSD1 inhibition. Besides, the compounds exhibited weak antiproliferative activity against the tested cancer cells, suggesting the low toxicity. Mechanistic studies showed that compound **29b** concentration-dependently induced accumulation of LSD1 substrates H3K4me1/2 in LSD1 overexpressed MGC-803 cells and also inhibited metastasis of MGC-803 cells in the transwell and wound healing assays. Taken together, compounds **26b** and **29b** are two promising LSD1 targeting lead compounds for further development and have therapeutic potentials.

Declaration of Competing Interest

The authors declare that they have no competing financial interests

Table 2
Cellular antiproliferative activity of compounds **26b** and **29b** against the tested cancer cell lines.

Compound	Inhibition [%]			
	PC-3	MCF-7	MGC-803	SGC-7901
26b	25.17	37.26	16.30	41.95
29b	21.84	33.65	24.54	27.68

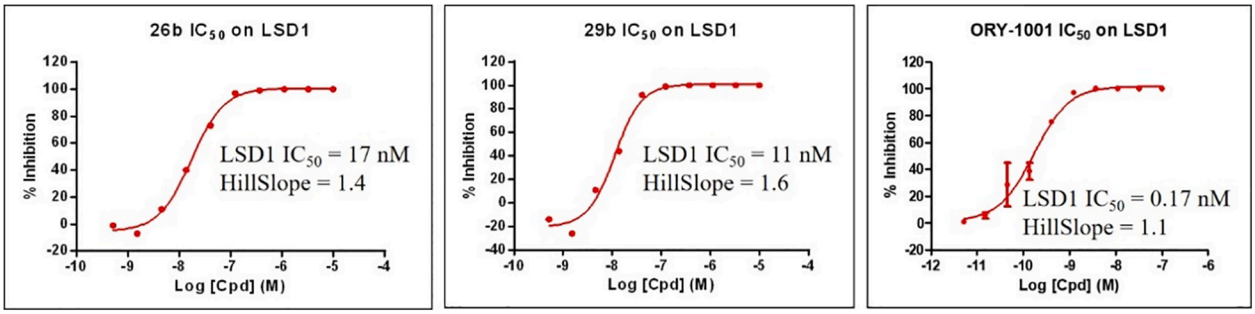


Fig. 2. Inhibition cures of compounds **26b**, **29b** and ORY-1001 against LSD1.

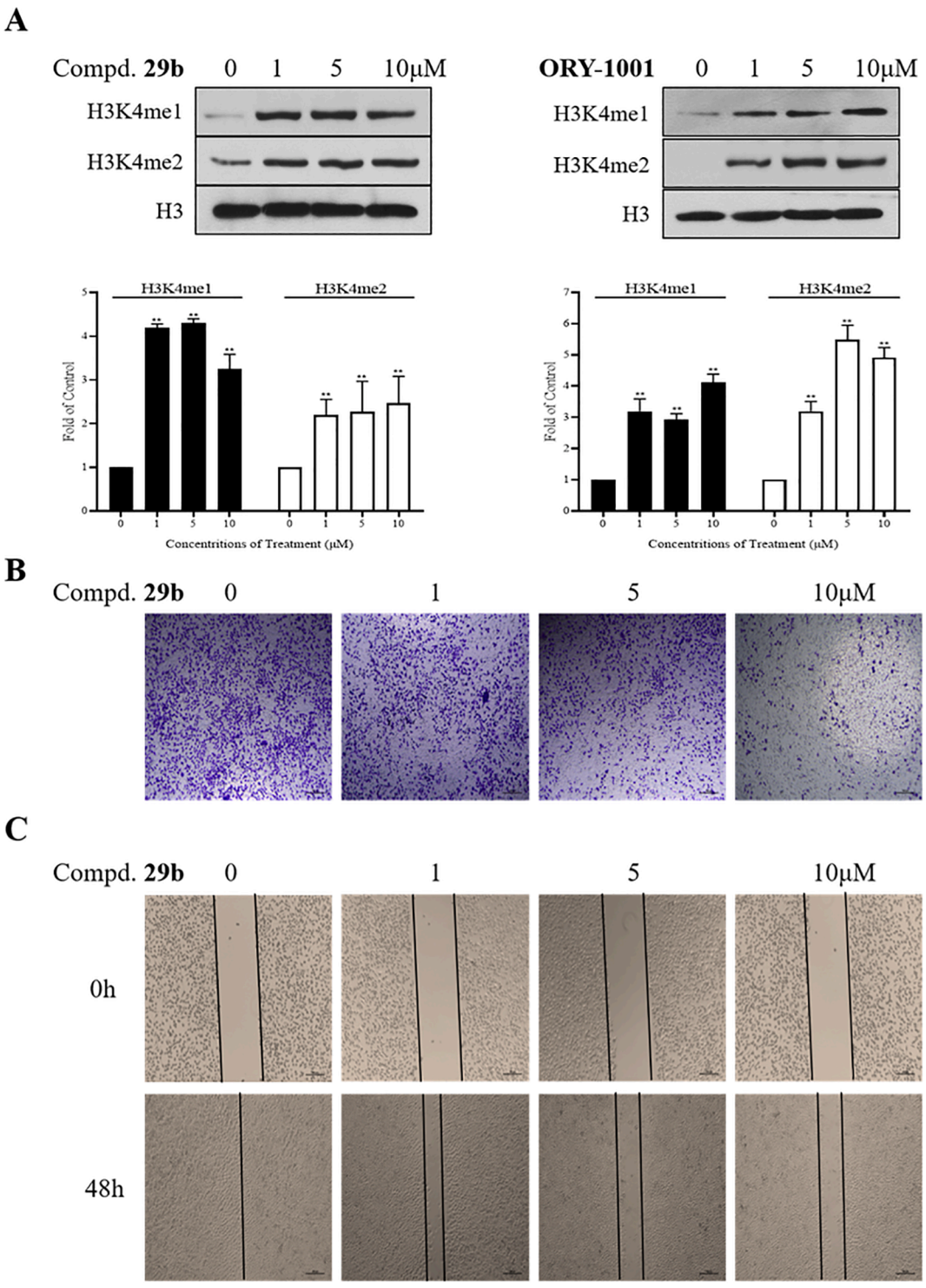


Fig. 3. Cellular effects of compound **29b** against MGC803 cells. (A) Expression of LSD1 substrates, H3K4me and H3K4me2, after treatment with compound **29b** for 72 h in MGC-803 cells. H3 was used as the loading control; (B) Migration assay; (C) Wound healing assay. Data were shown as mean ± SD. $^{**}p < 0.01$ was considered statistically significant compared with the control. All the experiments were performed at least three times and a representative result was shown.

or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127993.

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