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## Triazole-dithiocarbamate based, selective LSD1 inactivators inhibit gastric cancer cell growth, invasion and migration

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### Abstract

Lysine specific demethylase 1 (LSD1), the first identified histone demethylase, plays an important role in epigenetic regulation of gene activation and repression. The up-regulated LSD1's expression has been reported in several malignant tumors. In the current study, we designed and synthesized five series of 1, 2, 3-triazole-dithiocarbamate hybrids and screened their inhibitory activity toward LSD1. We found that some of these compounds, especially compound **26**, exhibited the most specific and robust inhibition of LSD1. Interestingly, compound **26** also showed potent and selective cytotoxicity against LSD1 overexpressing gastric cancer cell lines MGC-803 and HGC-27, as well as marked inhibition of cell migration and invasion, compared to **2-PCPA**. Furthermore, compound **26** effectively reduced the tumor growth bared by human gastric cancer cells in vivo with no signs of adverse side effects. These findings suggested that compound **26** deserves further investigation as a lead compound in the treatment of LSD1 overexpressing gastric cancer.

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**Supporting Information.** Characterization data for selected compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## INTRODUCTION

Epigenetic post-transcriptional modifications on histone, including acetylation, methylation, and phosphorylation, modulate gene activation and repression. Among these modifications, methylation and demethylation of lysine are dynamically regulated by a number of histone lysine methyltransferases (HKMTs) and histone lysine demethylases (HKDMs) including lysine specific demethylase 1 (LSD1). LSD1, the first characterized demethylase in 2004,<sup>1</sup> is a highly conserved flavin adenine dinucleotide (FAD)-dependent oxidative enzyme, containing amine oxidase domain. It demethylates mono-, di-methylated K4 and K9 of histone 3, as well as p53, E2F transcription factor 1 (E2F1) and DNA methyltransferases (DNMTs) and further regulates their downstream cellular function.<sup>2-7</sup> LSD1 has been reported to be overexpressed in many malignant tumors, including breast, colon, prostate, lung, gastric cancers and others.<sup>8-16</sup> Down regulation of LSD1 by RNAi or various kinds of inhibitors has been shown to effectively treat those cancers by inducing re-expression of aberrantly silenced genes.<sup>11, 12, 17-23</sup> Therefore, LSD1 has been considered an important and promising anticancer target.

As a member of monoamine oxidase (MAO) family, LSD1 utilizes a noncovalently bound FAD as its cofactor to oxidatively remove the methyl groups of its substrates.<sup>1</sup> MAO-A and MAO-B, another two members of MAO family, share the same mechanism and cofactor of LSD1 in the cleavage of the inactivated carbon-nitrogen bonds from their substrates.<sup>24</sup> MAO inactivators (Figure 1), such as pargyline (**1**), phenelzine (**2**) and tranylcypromine (**3**, **2-PCPA**), have been reported to function as non-selective LSD1 inhibitors.<sup>25</sup> In addition, **2-PCPA** derivatives, peptides and polyamine analogs have been synthesized as LSD1 inhibitors.<sup>12, 17, 18, 26-33</sup> However, only three new classes of LSD1 chemical inhibitors, including amidoxime based compounds,<sup>22</sup> amidino-guanidinium compounds,<sup>34</sup> and phenyl oxazoles,<sup>19</sup> have been reported during the past two years. Highly selective LSD1 inhibitors with strong toxicity toward cancer cells and less or no side effects on normal cells remain to be identified.

The azole heterocycles-pyrazole, including thiazole, imidazole, pyrrole, oxadiazole and triazole-have attracted more attention by medicinal chemists for many years due to their numerous biological activities,<sup>35, 36</sup> especially their MAO inhibitory effect.<sup>37-43</sup> Among these azole heterocycles, 1, 2, 3-triazole was mainly used based on its synthetic accessibility by click chemistry as well as its capacity for binding of biomolecular targets. Recently, several inhibitors toward MAO-A were developed accordingly.<sup>44, 45</sup> Furthermore, click chemistry has been widely used for synthesizing other inhibitors against epigenetic enzymes, such as HDAC.<sup>46, 47</sup> Hence, in this study, 1, 2, 3-triazole was chosen as a part of our target compound skeleton.

Dithiocarbamates was selected due to their inhibitory activities against fungal, bacteria and malignant cancer.<sup>48-50</sup> Disulfiram (DSF), as a supporting treatment of chronic alcoholism, is commercially available, and recently it has been reported as P-glycoprotein efflux pump modulator with antifungal potential.<sup>51</sup> Furthermore, when disulfiram creates complexes with metals, it becomes a proteasome inhibitor and acts as a promising approach for anticancer therapy.<sup>51</sup> BO-3482, a novel dithiocarbamate-containing carbapenem with activity against MRSA (Methicillin-Resistant Staphylococcus Aureus), has been tested in preclinical trail.<sup>52, 53</sup> Meanwhile, we have previously reported a pool of novel butenolides-containing dithiocarbamates with good anticancer activity.<sup>54</sup> Therefore, based on an existing pool of triazole-containing dithiocarbamates,<sup>55</sup> a library with eighty-four 1, 2, 3-triazole-dithiocarbamate hybrids was synthesized by the click chemistry approach. Their anti-LSD1 activity and cytotoxicities were then determined. We found that compared to **2-PCPA**, a non-selective LSD1 inhibitor, triazole-dithiocarbamate-based LSD1 inhibitors, especially

compound **26**, are more potent and exhibits selective inhibition of the growth of LSD1 over-expressing gastric cancer cell lines. Compound **26** also impaired cell migration and invasion and significantly inhibited tumor growth *in vivo*. These findings indicate that triazole-dithiocarbamate based LSD1 inhibitors represent a novel class of LSD1 inhibitors against LSD1 overexpressing gastric cancer.

## CHEMISTRY

Alkyne intermediates **4a–4j** were synthesized as shown in Scheme 1. Commercially available amines were treated with CS<sub>2</sub> and propargyl bromide in the presence of Na<sub>3</sub>PO<sub>4</sub>·12H<sub>2</sub>O in one pot to provide **4a–4j** (Figure 2).

Azides **5a** and **5e–5g** (Figure 3) were obtained from commercially available bromides by direct azide displacement. Azides **5b** were prepared using classical diazotation-azidation protocols.<sup>56</sup> The synthesis of azide intermediates **5c**, **5d**, **5h** and **5i** (Figure 3) is shown in Scheme 2. Substituted phenethyl alcohol was esterified to tosylate **6**, which was converted to azide derivative **5c** by reacting with sodium azide in acetonitrile. Hydroxy coumarin was treated with 1, 2-dibromoethane or tosyl chloride and then with sodium azide to acquire **5d** and **5i**, respectively. Azide derivative **5h** was prepared by a sodium azide substitution of **10**, obtained from starting material **8** from the reported procedures.<sup>57, 58</sup>

The 1, 2, 3-triazole-dithiocarbamate hybrids **12–72** were synthesized through a Huisgen 1, 3-dipolar cycloaddition between alkynes **4a–j** and azides **5a–i** using copper sulfate and sodium ascorbate in aqueous THF (Scheme 3). The synthesis of 1, 2, 3-triazole-dithiocarbamate hybrids **73–95** is shown in Scheme 4. **22–28** and **38–40** were deprotected using trifluoroacetic acid in dichloromethane to afford compounds **73–82**. Compounds **83–92** were obtained by reaction of **73–78** and **81–82** with corresponding chloroformic ester under basic condition. Compounds **73–75** and **77** were treated with acetyl chloride and Et<sub>3</sub>N to obtain **93–95**.

## RESULTS AND DISCUSSION

### Identification of LSD1 Inhibitors and structure-activity relationship (SAR) Studies

To determine the inhibitory activity of this compound library against LSD1, we generated a LSD1 recombinant expressing vector containing human LSD1 cDNA. The expression of recombinant LSD1 was then induced in *Escherichia Coli* (*E. Coli*) and purified as published.<sup>18</sup> The demethylation activity of the recombinant LSD1 was further determined by a fluorescence-based method, using synthesized H3K4me<sub>2</sub> as a substrate.<sup>32</sup> All the compounds synthesized in this study were examined *in vitro* for their inhibitory effect on LSD1 activity, **2-PCPA** was chosen as a positive control. The results were summarized as Table 1–5.

The *in vitro* inhibitory activity results of compounds **12–40** against LSD1 were determined initially and shown in Table 1. With the exception of compounds **29–31** and **36**, all the compounds bearing a carbamate moiety exhibit moderate to good potency with IC<sub>50</sub> values ranging from 2.1 to 88.9 μM. Among them, compound **26** shows the most potent activity to LSD1, with an IC<sub>50</sub> of 2.1 μM, which is 14 times higher than that of **2-PCPA**. Moreover, compounds **22–24**, **27** and **40** are more potent than **2-PCPA**, with IC<sub>50</sub> values in the single- or double-digit micromolar range. During the SAR studies, we found that the substitution on the phenyl ring was important for the *in vitro* LSD1 inhibitory activity showing over 35-fold activity loss, when the methyl group was replaced with the hydroxyl group (compound **26** vs **33**). We also observed that the *para* substitution was preferential: the *p*-CH<sub>3</sub> derivative compound **26** was more potent against LSD1 than the *m*-CH<sub>3</sub> derivative **28** (IC<sub>50</sub> = 43.3

$\mu\text{M}$ ) and the *o*-CH<sub>3</sub> derivative **29** without any detectable activity. The nitrogen of the piperidine was essential. When the nitrogen was replaced with a methylene or an oxygen atom (**12–16**, **17–21**), their inhibitory activities to LSD1 were completely lost.

To determine whether the length of carbon tether between the triazole ring and the benzene ring might affect the compounds' binding of LSD1, compounds **41–46** were synthesized and their LSD1 inhibitory activities were shown in Table 2. Either extension or reduction of the carbon tether length by one carbon, a complete loss of potency was observed. Those results suggested that the linker between the benzene ring and the triazole plays an important role in their activities.

The importance of benzene ring in the LSD1 inhibitory activity was also explored (Table 3). Replacing the phenyl scaffold of compound **26** with coumarin ring (**47**), naphthalene ring (**48**), quinolinone ring (**49**), benzoxazole ring (**50**) or butenolide ring (**51**) led to a complete loss of activity or very weak binding affinity, indicating the significance of the benzene ring in retaining their activity.

Furthermore, the importance of substituents on the N-atom (Table 4) was investigated. Changing the *t*-butyloxycarbonyl group to alkyl chain methyl, ethyl, benzyl or hydroxyethyl resulted in inactive compounds **53–63**. Replacing the *t*-butyloxycarbonyl group by phenyl (as in **64–66**), 2-pyrimidinyl (as in **67–68**) or mesyl (as in **69–71**) caused dramatic loss of activity. Removing the *t*-butyloxycarbonyl group was clearly detrimental for the inhibition of LSD1, such as, compound **26** (2.1  $\mu\text{M}$ ) compared to **77** (28.9  $\mu\text{M}$ ) and **73** (17.3  $\mu\text{M}$ ). These modifications and SAR studies revealed that the *t*-butyloxycarbonyl group is critical for their inhibitory activity.

We next synthesized compounds **83–95** to explore the bulk of carbamate moiety and the effect of the oxygen atom. The results were shown in Table 5. Changing the *tert*-butyl carbamates to benzyl carbamates (**83–90**) dramatically decreased the inhibitory activity, by comparing **87–88** with **26–27**. Compared to compound **26**, the ethyl carbamate **91** and isopropyl carbamate **92** showed 8.5- to 7.5-fold loss of activity in their IC<sub>50</sub> values, respectively. This finding indicated that steric hindrance of the carbamoyl moiety was of pivotal importance in their activity. The role of the oxygen atom was investigated by preparing compounds **93–95**, in which the oxygen atom was replaced with a methylene group. These compounds showed moderate activity with IC<sub>50</sub> ranging from 25.3–47.1  $\mu\text{M}$ , which is consistent with the results described above that the *t*-butyloxycarbonyl group is critical for the inhibitory activity.

### ***In vitro* inhibition properties of compound 26 to the recombinant LSD1 and its homologues: LSD2, MAO-A and MAO-B**

Having identified compound **26** as a highly potent LSD1 inhibitor, we further determined the dissociation constant ( $K_d$ ) of compound **26** by microscale thermophoresis (MST) experiment. The  $K_d$  values for compound **26** and **2-PCPA** were 0.35 and 46.3  $\mu\text{M}$  (Figure 4A, B), respectively, indicating the robust binding affinity of compound **26** to LSD1, compared to **2-PCPA** (Figure 4A, B). Then, the inhibitory properties of compound **26** were characterized. We found that, during 60 min, the inhibition of compound **26** to LSD1 activity with three different concentrations (0.5, 2.5 and 12.5  $\mu\text{M}$ ) exhibit time independent manner (Figure 4D). To test the reversibility of compound **26** for LSD1, a dilution assay was used. Our analysis suggested that dilution of the LSD1/compound **26** mixture by 80 folds resulted in the recovery of LSD1 activity, which means the molecule may interact noncovalently with the enzyme. However, in the presence of the covalently binding inhibitor **2-PCPA**, LSD1 activity cannot be recovered after dilution (Figure 4E). These results

indicate the reversibility of compound **26**, compared to **2-PCPA**. Dialysis experiment was also performed against hepes buffer after LSD1 was inactivated by high concentration of compound **26**. After the dialysis, the activity can be restored either (Figure 4F), which further support the reversible inhibitory of compound **26**. Next, the competitive analysis was performed to test the hypothesized binding sites of compound **26** on LSD1. With classic Lineweaver-Burk plots,<sup>59</sup> we characterized that compound **26** was a non-competitive inhibitor over LSD1 substrate, H3K4me2 (Figure 4G), but a competitive inhibitor over LSD1 cofactor FAD (Figure 4H). These results indicate that compound **26** may penetrate into the cavity in LSD1 where FAD stands and reduce the recombinant activity. Another dialysis experiment was done to verify the FAD ejection with compound **26** treatment on LSD1. The inhibitor, FAD and LSD1 were incubated and dialyzed against the hepes buffer containing the same concentration of inhibitor. Then, the amount of FAD in the dialysis tubes was monitored at 468nm with UV/VIS spectrophotometer. After dialysis, the amount of FAD in the compound **26** dialysis tube was much less than that in control group (Data not shown), indicating that the FAD may be ejected with the treatment of compound **26**. The  $K_d$  value of FAD is 0.182  $\mu$ M (Figure 4C), smaller than that of compound **26**, indicating that FAD, as the cofactor of LSD1, binds to this enzyme more tightly than compound **26**. However, at a relative higher concentration, compound **26** may still exhibit competitive inhibition against FAD. It is not so common to create a FAD competitor. But, there are some published examples on FAD competitors. Quinine and mepacrine (atrabrine) were stated to inhibit D-aminoacid oxidase by competing with FAD.<sup>60</sup> Chlorpromazine (CPZ), a dopamine D2 receptor antagonist introduced in the treatment of schizophrenia in the early 1950s, acts as a FAD-competitive inhibitor of human D-amino acid oxidase (hDAAO) with similar structure.<sup>61</sup> **K001** that competes for FAD binding, inhibits cryptochrome (CRY) ubiquitylation and lengthens the circadian cycle.<sup>62</sup> There are some other papers published on FAD ejectors.<sup>63, 64</sup> EN460, an inhibitor of endoplasmic reticulum oxidation 1 (ERO1), was reported in 2010.<sup>64</sup> This compound exhibits selectivity for ERO1 and displaces of bound FAD from the active site of the enzyme. Nevertheless, the structure of EN460 is different from that of FAD. Furthermore, several other compounds were obtained with virtual screening for a lead, which would compete with FAD.<sup>63</sup> Hence, although the structure of compound **26** is not similar with FAD, it is still possibly for it to acts as FAD competitor or FAD ejector. On the other hand, compound **26** may also bind to an allosteric site of LSD1,<sup>65, 66, 67</sup> resulting in an apparent change in binding affinity of another ligand at a distinctly different site. Furthermore docking analysis also illustrated the interaction between compound **26** and LSD1.

Activity of LSD1 and LSD2 recombinant was evaluated by quantifying the demethylase byproduct  $H_2O_2$  with Amplex Red method coupled with HRP. Activity of MAO-A and MAO-B was evaluated by the commercialized kit. Data are mean $\pm$ SD. All experiments were repeated at least three times.

As LSD1 belongs to the monoamine oxidase (MAO) family, the inhibitory effects of compound **22** and **26** to its homologies: MAO-A and MAO-B were also examined using commercially available kits. Compared to **2-PCPA**, compounds **22** and **26** had no significant effects on MAO-A and MAO-B activities (Table 6). Recently, another FAD dependent lysine demethylase, named LSD2, was identified with 45% sequence identity with LSD1.<sup>68, 69</sup> Therefore, the LSD2 recombinant encoding 22-822AA was also generated from *E. Coli* and the purified recombinant was then applied for its activity assay. As shown in Table 2, compound **22** and **26** showed poor inhibitory effect against LSD2 recombinant. These findings indicated the high selectivity of this class of inhibitors on LSD1 *in vitro*.



## Docking and Molecular Dynamics (MD) Simulations

Predictions of the binding modes involve a comprehensive evaluation of the center structures of the clusters generated from a Perron-Cluster Cluster Analysis (PCCA) using the following three criteria: (1) the protein-ligand interaction energy obtained from the PCCA analysis; (2) the number of hydrogen bonds between the protein and the ligand calculated using a distance (donor-acceptor) and angle cutoff of 3 Å and 20°, respectively; (3) the stability of the ligand in the binding site during MD simulations. Using the above procedure, two types of binding modes were identified for the ligand (Figure 5).

The binding modes predicted that the carbonyl oxygen (next to the tri-methyl group) is able to form hydrogen bonds to the backbone nitrogen of either ALA331 (Figure 5A) or both MET332 and VAL333 (Figure 5B). The triazole ring is also favorably positioned to form hydrogen bonds to the backbone nitrogen of ARG316 (Figure 5A). Furthermore, ALA331, MET332, VAL333 and ARG316 are part of the pocket that surround the isoalloxazine moiety of FAD.<sup>70</sup> Occupation of these amino acids may result in the ejection of FAD. To further assess the dynamic stabilities of the predicted binding poses, short (1 ns) MD simulations were also performed on the binding poses of the two ligands. Overall, we found that both binding modes were well maintained during the short MD simulations for the ligands, which is in line with the experimental data showing that compound **26** stand in the cavity of LSD1 belong to the FAD-binding subdomain, while Arg 316 is one of the binding sites of FAD.<sup>71</sup> These results further support our findings about the competitive inhibition of compound **26** over FAD, but specific mechanism on how does compound **26** act on LSD1 still need to be clarified in the further structure analysis.

## Effects of compounds **22** and **26** on LSD1 activity in cultured gastric cancer cells and their cytotoxicities

To determine whether compounds **22** and **26** are cell active LSD1 inhibitors, the effect of these compounds on the methylation levels of LSD1 substrates H3K4 and H3K9 were analyzed in MGC-803 cells. We found that the amounts of H3K4me/me2 and H3K9me2 were dose dependently elevated after treatment of MGC-803 cells with these two compounds (Figure 6A, B). These results validated that compounds **22** and **26** can decrease LSD1 activity at the cellular level. To further determine the specificity of compounds **22** and **26** at the cellular level, the expression level of H3K4me3 that is the substrate of histone demethylase JARID1, was also evaluated. Figures 6A and B show that compounds **22** and **26** did not affect the expression of H3K4me3. These results suggested that compounds **22** and **26** are specific and cell active LSD1 inhibitors.

To further determine whether compounds **22** and **26** are potential candidates for chemotherapeutic agents against gastric cancer, the *in vitro* cytotoxicities of compounds **22** and **26** were evaluated by MTT method. Both compounds exhibited strong cytotoxicity against human gastric cancer cell lines MGC-803 and HGC-27 with higher LSD1 expression (IC<sub>50</sub> of compounds **22** and **26** for MGC-803 line are 1.26 μM and 0.89 μM respectively; IC<sub>50</sub> of compounds **22** and **26** for HGC-27 cell line are 1.56 μM and 1.13 μM, respectively) (Figures 7A, B, C, D). However, both of them did not show any marked effects on normal gastric epithelial cell line GES-1 and gastric cancer cell line SGC-7901 and both with lower LSD1 expression (IC<sub>50</sub> of compounds **22** and **26** for SGC-7901 cell line are 69.5 μM and 89.5 μM respectively; IC<sub>50</sub> of compounds **22** and **26** for GES-1 cell line are 50.26 μM and 45.26 μM respectively) (Figure 7A, B, C, D, E). siRNA mediated LSD1 knock down (Figure 7F) in MGC-803 cells also resulted in a loss of cell viability (Figure 7G). These findings implicated that the cytotoxicities of compounds **22** and **26** on gastric cancer cells may be associated with their specific inhibition to the enhanced LSD1 expression in those cells.

## Effect of Compound 26 on Apoptosis, Cell migration, Invasion

To explore cytotoxicity of **26** in MGC-803 cells, cell apoptosis was investigated with Hoechst 33258 staining. After 48h incubation with **26** at indicated concentrations, characteristic apoptotic morphological changes were observed by fluorescence microscope, including cell rounding, chromatin shrinkage and formation of apoptotic bodies (Figure 8A). The apoptotic analysis was also performed with Annexin V-FITC/PI double staining and quantitated by flow cytometry, compound **26** treatment of MGC-803 cells dose dependently increased the percentage of the apoptotic population up to 12.6%, 31.7% and 44.7%, respectively, compared to control (7.9%) (Figure 8B, C). As LSD1 shows a strong preference to reverse K370me2 of p53, which inhibits its association with the coactivator 53BP1 (p53-binding protein 1) through tandem Tudor domains in 53BP1 and represses p53 function,<sup>5</sup> we suppose that **26** may prevent the demethylation at K370me2 of p53 by LSD1 and promote p53-mediated apoptosis.

In addition, MGC-803 cell migration was evaluated by wound healing and transwell experiments. Cell invasion capacity was determined by matrigel coated transwell experiment following a reported procedure.<sup>72</sup> Microphotographs showed that untreated gastric cancer MGC-803 cells filled most of the wounded area two days after scratching the cell monolayer, whereas treatment with indicated doses of compound **26** markedly suppressed repairment of the wound (Figure 9A). The inhibitory effect of compound **26** on repopulation of the wounded area was not due to decreased proliferation, because the highest concentration (0.25  $\mu$ M) of compound **26** we used in this assay cannot inhibit the cell proliferation as we discussed in Figure 8. Compound **26** also significantly inhibited the cell migration and invasion even at low concentration (0.02  $\mu$ M) (Figure 9B and C), so did the siRNA mediated LSD1 knock down, when compared to the positive control 100 $\mu$ M **2-PCPA** (Figure 9B and C). These results indicate that the effect of compound **26** on cell migration and invasion is much more powerful than **2-PCPA**. LSD1 can form a complex with snai1 to act at the E-Box domain of E-Cadherin and restrain the expression of E-cadherin for promoting cell migration and invasion.<sup>53, 54</sup> After incubation with compound **26**, the protein levels of E-Cadherin were markedly elevated, without significant alteration of the total levels of LSD1 and snai1, compared to those on control (Figure 9D, E). To further determine the effects of compound **26** on the complex formation between LSD1 and snai1, co-immunoprecipitation was performed, using LSD1 antibody as a bait. After compound **26** treatment for 48h, the binding of LSD1 to snai1 were significantly decreased, which is the same as that by the positive control **2-PCPA** (Figure 9F, G). These results indicated that the enhanced expression of E-Cadherin by compound **26** may be associated with the decreased interaction of LSD1 and snai1, and further clarified the inhibitory mechanism on the cell migration and invasion by this compound.

### *In vivo* anti-tumor study

We next examined the effect of compound **26** on tumor growth *in vivo* in a *xenograft* model. Xenograft tumors were generated by subcutaneous implantation of MGC-803 cells into nude mice. After the treatment, body weights of the mice were monitored and their tumor sizes were measured and recorded every 3 days (Figure 10A). Compound **26** treatment at a dose of 20mg/kg significantly inhibited the growth of tumor over time and reduced tumor weight by 68.5% (Figures 10A, B and C). There were no apparent body weight loss during the treatment (Figure 10D). These data indicate that compound **26** was efficacious in inhibiting the growth of LSD1 overexpressing gastric tumor *in vivo*, but no obvious global toxicity.



## CONCLUSION

In summary, we have synthesized and identified a novel class of triazole-dithiocarbamate based, selective LSD1 inhibitors. Some of these triazole-dithiocarbamates, especially compound **26**, exhibit reversible and FAD competitive LSD1 inhibition. To our best knowledge, compound **26** is the first identified highly selective LSD1 inhibitor with the ability to inhibit cell migration and invasion at lower concentrations. Our findings indicate that the lead triazole-dithiocarbamate based LSD1 inhibitor may be developed as a novel anti-cancer epigenetic drug to target gastric cancer with overexpression of LSD1.

## METHODS

### General Methods for Chemistry

Reagents and solvents were purchased from commercial sources and were used without further purification. Melting points were determined on a X-5 micromelting apparatus and.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer respectively. IR spectra were recorded on a Nicolet iS10 infrared spectrometer. High resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer. The purity of all biologically evaluated compounds was determined to be >95% by reversephase high performance liquid chromatography (HPLC) analysis. HPLC measurement was performed with a Phenomenex column (C18, 5.0  $\mu\text{m}$ , 4.6 mm  $\times$  150 mm) on Dionex UltiMate 3000 UHPLC instrument from ThermoFisher. The signal was monitored at 254nm with a UV detector. A flow rate of 0.5 mL/min was used with mobile phase of MeOH in H<sub>2</sub>O (80:20, v/v).

**General procedure for the synthesis of alkynes 4a-j**—CS<sub>2</sub> (2.28 g, 30 mmol) was added drop wise to the solution of *tert*-butyl piperazine-1-carboxylate (1.86g, 10 mmol) and Na<sub>3</sub>PO<sub>4</sub>·12H<sub>2</sub>O (2.28 g, 6 mmol) in acetone (40 mL). The reaction mixture was stirred at room temperature for 0.5 h. Then propargyl bromide (1.31 g, 11 mmol) was added to the mixture drop wise, the reaction mixture was stirred at room temperature for another 0.5 h. Then, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure, the residue was dissolved in EtOAc (50 mL), washed with water (50 mL), brine (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to afford alkyne **4j**. Prop-2-ynyl 4-(2-hydroxyethyl)piperazine-1-carbodithioate (**4c**): Yield 82.2%, white solid. Mp: 96–97°C;  $^1\text{H}$  NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  4.36 (brs, 2H), 4.12 (d,  $J$  = 2.7 Hz, 2H), 3.96 (brs, 2H), 3.69 (t,  $J$  = 5.3 Hz, 2H), 2.64 (m, 6H), 2.27 (t,  $J$  = 2.7 Hz, 2H); HRMS (ESI) calcd for C<sub>10</sub>H<sub>17</sub>N<sub>2</sub>OS<sub>2</sub> [M + H]<sup>+</sup>: 245.0782, found: 245.0781.

*tert* Butyl 4-((prop-2-ynylthio)carbonothioyl)piperazine-1-carboxylate (**4j**): Yield 92%, white solid. Mp: 87–88°C.  $^1\text{H}$  NMR (400 MHz, Acetone-d<sub>6</sub>, ppm):  $\delta$  4.28 (brs, 2H), 4.14 (d,  $J$  = 2.7 Hz, 2H), 4.00 (brs, 2H), 3.58 (brs, 4H), 2.78 (t,  $J$  = 2.7 Hz, 1H), 1.46 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  195.16, 154.39, 80.68, 78.19, 71.82, 28.36, 26.00; HRMS (ESI) calcd for C<sub>13</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub> [M + H]<sup>+</sup>: 301.1044, found: 301.1046.

**General procedure for the synthesis of compounds 12–72**—In a round-bottom flask equipped with a magnetic stirred bar, alkyne derivatives **4** (5 mmol), azide derivatives **5** (5.5 mmol), CuSO<sub>4</sub>·5H<sub>2</sub>O (62mg, 0.25 mmol), sodium ascorbate (100 mg, 0.5 mmol), THF (20 mL) and H<sub>2</sub>O (20 mL) were added. The resulting mixture was stirred at room temperature for about 2 h. The disappearance of compounds **4** was monitored by TLC (silica gel, PE:EtOAc = 3:1). After the reaction, water (40 mL) was added and the reaction mixture was extracted with EtOAc (3 $\times$ 40 mL). The combined organic layer was washed with brine (100 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to afford the crude

products. The crude products were recrystallized from acetone to yield the pure products **12–72**.

tert Butyl 4-(((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonothioyl)-piperazine-1-carboxylate (**22**): Yield 79.0%, white solid; Mp: 109–110°C; IR (KBr,  $\text{cm}^{-1}$ )  $\nu$ : 3447, 2979, 1693, 1494, 1478, 1424, 1279, 1167, 1012, 986, 932, 791, 757, 695;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.66 (s, 1H), 7.09–7.38 (m, 4H), 5.55 (s, 2H), 4.69 (s, 2H), 4.29 (brs, 2H), 3.91 (brs, 2H), 3.54 (t,  $J = 5.1$  Hz, 4H), 1.47 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  196.42, 161.72, 159.26, 154.41, 144.00, 130.89, 130.81, 130.51, 130.48, 124.82, 124.78, 122.96, 121.98, 121.84, 115.92, 115.71, 80.63, 47.66, 47.61, 31.84, 28.34;  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ )  $\delta$  -118.03; HRMS (ESI) calcd for  $\text{C}_{20}\text{H}_{27}\text{FN}_5\text{O}_2\text{S}_2$   $[\text{M} + \text{H}]^+$ : 452.1590, found: 452.1598.

tert Butyl 4-(((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonothioyl)-piperazine-1-carboxylate (**23**): Yield 81.5%, white solid. Mp: 171–172°C; IR (KBr,  $\text{cm}^{-1}$ )  $\nu$ : 3482, 3139, 2974, 1690, 1470, 1420, 1281, 1167, 1051, 994, 824, 781, 541;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  7.59 (s, 1H), 7.04–7.27 (m, 4H), 5.46 (s, 2H), 4.68 (s, 2H), 4.31 (brs, 2H), 3.90 (brs, 3H), 3.53 (s, 4H), 1.47 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  196.40, 164.07, 161.60, 154.40, 130.49, 130.45, 129.96, 129.87, 116.20, 115.98, 80.66, 53.42, 31.78, 28.34;  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ )  $\delta$  -112.68; HRMS (ESI) calcd for  $\text{C}_{20}\text{H}_{27}\text{FN}_5\text{O}_2\text{S}_2$   $[\text{M} + \text{H}]^+$ : 452.1590, found: 452.1588.

tert Butyl 4-(((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonothioyl)-piperazine-1-carboxylate (**24**): Yield 78.7%, white solid. Mp: 105–106°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  7.70 (s, 1H), 7.46 (dd,  $J_1 = 1.4$  Hz,  $J_2 = 7.8$  Hz, 1H), 7.23–7.33 (m, 2H), 7.18 (dd,  $J_1 = 1.6$  Hz,  $J_2 = 7.4$  Hz, 1H), 5.62 (s, 2H), 4.70 (s, 2H), 4.28 (brs, 2H), 3.93 (brs, 2H), 3.54 (t,  $J = 5.2$  Hz, 4H), 1.47 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz, Acetone- $d_6$ , ppm):  $\delta$  195.68, 154.33, 143.01, 133.30, 133.16, 130.60, 130.31, 129.74, 127.71, 123.79, 79.78, 50.99, 31.63, 27.63; HRMS (ESI) calcd for  $\text{C}_{20}\text{H}_{27}\text{ClN}_5\text{O}_2\text{S}_2$   $[\text{M} + \text{H}]^+$ : 468.1295, found: 468.1292.

tert Butyl 4-(((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonothioyl)-piperazine-1-carboxylate (**26**): Yield 81.9%, white solid. Mp: 182–183°C; IR (KBr,  $\text{cm}^{-1}$ )  $\nu$ : 3454, 2975, 1682, 1457, 1422, 1224, 1078, 994, 933, 867, 772, 524;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  7.55 (s, 1H), 7.16–7.21 (m, 4H), 5.43 (s, 2H), 4.67 (s, 2H), 4.29 (brs, 2H), 3.91 (brs, 2H), 3.54 (t,  $J = 5.2$  Hz, 4H), 2.35 (s, 3H), 1.47 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  196.49, 154.41, 138.67, 131.57, 129.75, 128.09, 80.64, 54.01, 31.91, 28.35, 21.15; HRMS (ESI) calcd for  $\text{C}_{21}\text{H}_{30}\text{N}_5\text{O}_2\text{S}_2$   $[\text{M} + \text{H}]^+$ : 448.1841, found: 448.1840.

tert Butyl 4-(((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonothioyl)-piperazine-1-carboxylate (**27**): Yield 85.8%, white solid. Mp: 129–130°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  7.54 (s, 1H), 7.21 (d,  $J = 8.7$  Hz, 2H), 6.88 (d,  $J = 8.7$  Hz, 2H), 5.41 (s, 2H), 4.67 (s, 2H), 4.29 (brs, 2H), 3.90 (brs, 2H), 3.80 (s, 3H), 3.51 (t,  $J = 5.2$  Hz, 4H), 1.47 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  196.47, 159.89, 154.40, 143.86, 129.62, 126.59, 122.52, 114.44, 80.61, 55.34, 53.70, 31.90, 28.35; HRMS (ESI) calcd for  $\text{C}_{21}\text{H}_{30}\text{N}_5\text{O}_3\text{S}_2$   $[\text{M} + \text{H}]^+$ : 464.1790, found: 464.1794.

tert Butyl 4-(((1-(2-bromobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonothioyl)-piperazine-1-carboxylate (**32**): Yield 79.0%, white solid. Mp: 129–130°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  7.71 (s, 1H), 7.62 (dd,  $J_1 = 1.1$  Hz,  $J_2 = 7.9$  Hz, 1H), 7.32 (td,  $J_1 = 1.1$  Hz,  $J_2 = 7.5$  Hz, 1H), 7.24 (td,  $J_1 = 1.5$  Hz,  $J_2 = 7.8$  Hz, 1H), 7.12 (dd,  $J_1 = 1.5$  Hz,  $J_2 = 7.6$  Hz, 1H), 5.62 (s, 2H), 4.69 (s, 2H), 4.30 (brs, 2H), 3.92 (brs, 2H), 3.54 (t,  $J = 4.8$  Hz, 4H), 1.47 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 196.4, 154.4, 134.2, 133.2, 130.3,

130.2, 128.2, 123.4, 80.6, 53.8, 31.9, 28.4; HRMS(ESI) calcd for  $C_{20}H_{27}BrN_5O_2S_2$   $[M + H]^+$ : 512.0790, found: 512.0793.

**General procedure for the synthesis of compounds 73–82**— $CF_3COOH$  (4.56g, 40mmol) was added to a solution of **22** (0.90g, 2mmol) in  $CH_2Cl_2$  (20mL) at  $0^\circ C$ . The reaction mixture was warmed to room temperature and stirred at the same temperature for about 8 h. The disappearance of compounds **22** was monitored by TLC (silica gel,  $CH_2Cl_2:CH_3OH = 8:1$ ). The reaction mixture was concentrated under vacuum, the residue was dissolved in  $CH_2Cl_2$  (20 mL), washed with saturated  $NaHCO_3$  (50 mL), brine (50mL), dried over anhydrous  $Na_2SO_4$  and concentrated under vacuum to afford compounds **73**, which were used in the next reaction without further purification.

(1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**73**): Yield 96.7%, white solid. Mp:  $93-94^\circ C$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta$  7.67 (s, 1H), 7.34–7.40 (m, 1H), 7.24–7.26 (m, 1H), 7.11–7.18 (m, 2H), 5.55 (s, 2H), 4.69 (s, 2H), 4.30 (brs, 2H), 3.93 (brs, 2H), 2.96 (t,  $J = 4.8$  Hz, 4H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , ppm):  $\delta$  195.81, 161.72, 159.26, 144.27, 130.88, 130.80, 130.50, 130.47, 124.82, 124.79, 122.98, 122.00, 121.86, 115.93, 115.72, 47.66, 47.61, 45.58, 31.76; HRMS (ESI) calcd for  $C_{15}H_{19}FN_5S_2$   $[M + H]^+$ : 352.1066, found: 352.1064. 1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**78**): yield 94.0%. white solid. Mp:  $74-75^\circ C$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta$  7.56 (s, 1H), 7.18 (s, 4H), 5.43 (s, 2H), 4.67 (s, 2H), 4.31 (brs, 2H), 3.92 (brs, 2H), 2.96 (brs, 4H), 2.35 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , ppm):  $\delta$  195.82, 138.61, 131.59, 129.74, 128.08, 122.72, 53.96, 31.80, 29.68, 21.17; HRMS (ESI) calcd for  $C_{16}H_{22}N_5S_2$   $[M + H]^+$ : 348.1317, found: 348.1319.

(1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**79**): Yield 92.7%, white solid. Mp:  $95-96^\circ C$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta$  7.55 (s, 1H), 7.24 (d,  $J = 8.7$  Hz, 2H), 6.91 (d,  $J = 8.7$  Hz, 2H), 5.42 (s, 2H), 4.68 (s, 2H), 4.31 (brs, 2H), 3.95 (brs, 2H), 3.80 (s, 3H), 2.96 (t,  $J = 4.6$  Hz, 4H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , ppm):  $\delta$  195.8, 159.9, 144.1, 129.6, 126.6, 122.5, 114.5, 55.3, 53.7, 45.6, 31.8; HRMS (ESI) calcd for  $C_{16}H_{22}N_5OS_2$   $[M + H]^+$ : 364.1266, found: 364.1263.

**General procedure for the synthesis of compounds 83–92**—A mixture of **73** (0.70 g, 2 mmol),  $K_2CO_3$  (0.28 g, 2 mmol) and  $CbzCl$  (0.38 g, 2.2 mol) in  $CH_2Cl_2$  (20mL) was stirred at room temperature for 7 h. The disappearance of compounds **73** was monitored by TLC (silica gel, PE:Actone = 3:1). After the reaction,  $K_2CO_3$  was removed by filtration and the solvent was diluted with  $CH_2Cl_2$  (10 mL), washed with water (40 mL), brine (40 mL), dried over anhydrous  $Na_2SO_4$  and concentrated under vacuum to give the crude product, which was recrystallized from acetone to provide compound **83** as white solid.

Benzyl 4-(((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonothioyl) piperazine-1-carboxylate (**83**): Yield 74.6%, white solid. Mp:  $138-139^\circ C$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta$  7.66 (s, 1H), 7.08–7.37 (m, 9H), 5.54 (s, 2H), 5.15 (s, 2H), 4.69 (s, 2H), 4.30 (brs, 2H), 3.95 (brs, 2H), 3.61 (t,  $J = 5.2$  Hz, 4H);  $^{13}C$  NMR (100MHz,  $CDCl_3$ , ppm):  $\delta$  196.61, 161.73, 159.27, 155.04, 143.97, 136.21, 130.94, 130.85, 130.54, 130.51, 128.61, 128.30, 128.09, 124.84, 124.80, 123.04, 121.93, 121.79, 115.94, 115.73, 67.64, 47.72, 47.68, 43.02, 31.85; HRMS (ESI) calcd for  $C_{23}H_{25}FN_5O_2S_2$   $[M + H]^+$ : 486.1434, found: 486.1432.

Benzyl 4-(((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio) carbonothioyl) piperazine-1-carboxylate (**87**): Yield 81.8%, white solid. Mp:  $99-100^\circ C$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ , ppm):  $\delta$  7.55 (s, 1H), 7.14–7.37 (m, 9H), 5.44 (s, 2H), 5.16 (s, 2H), 4.67 (s, 2H), 4.29 (brs, 2H), 3.96 (brs, 2H), 3.59 (t,  $J = 5.1$  Hz, 4H), 2.35 (s, 3H);  $^{13}C$  NMR (100

MHz, CDCl<sub>3</sub>, ppm):  $\delta$  196.67, 155.04, 138.68, 136.21, 131.56, 129.76, 128.61, 128.31, 128.10, 122.73, 67.64, 54.02, 43.03, 31.95, 21.17; HRMS (ESI) calcd for C<sub>24</sub>H<sub>28</sub>N<sub>5</sub>O<sub>2</sub>S<sub>2</sub> [M + H]<sup>+</sup>: 482.1684, found: 482.1683.

Benzyl 4-(((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonthioyl)piperazine-1-carboxylate (**90**): Yield 82.0%, white solid. Mp: 134–135°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  7.63 (s, 1H), 7.35–7.37 (m, 5H), 6.47 (s, 2H), 5.40 (s, 2H), 5.15 (s, 2H), 4.68 (s, 2H), 4.28 (brs, 2H), 3.96 (brs, 2H), 3.83 (s, 3H), 3.82 (s, 6H), 3.62 (t, *J* = 5.0 Hz, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  196.60, 155.02, 153.69, 144.02, 138.30, 136.19, 130.08, 128.60, 128.30, 128.09, 122.82, 105.23, 67.64, 60.85, 56.25, 54.38, 43.01, 31.86; HRMS (ESI) calcd for C<sub>26</sub>H<sub>32</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub> [M + H]<sup>+</sup>: 558.1845, found: 558.1841.

Ethyl 4-(((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonthioyl)piperazine-1-carboxylate (**91**): Compound **91** was prepared from **77** and ethyl chloroformate using method above. Yield 77.4%, white solid. Mp: 100–101°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  7.57 (s, 1H), 7.17 (s, 4H), 5.44 (s, 2H), 4.70 (s, 2H), 4.31 (brs, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.93 (brs, 2H), 3.58 (s, 4H), 2.35 (s, 3H), 1.29 (t, *J* = 7.1 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  196.60, 155.25, 143.98, 138.70, 131.47, 129.77, 128.13, 122.82, 61.91, 54.08, 42.90, 31.85, 21.18, 14.65; HRMS (ESI) calcd for C<sub>19</sub>H<sub>26</sub>N<sub>5</sub>O<sub>2</sub>S<sub>2</sub> [M + H]<sup>+</sup>: 420.1528, found: 420.1527.

Isopropyl 4-(((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonthioyl)piperazine-1-carboxylate (**92**): Compound **92** was prepared from **77** and isopropyl chloroformate using method above. Yield 84.5%, Mp: 149–150°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  7.55 (s, 1H), 7.17 (s, 4H), 5.44 (s, 2H), 4.97 (m, 1H), 4.67 (s, 2H), 4.31 (brs, 2H), 3.93 (brs, 2H), 3.56 (s, 4H), 2.35 (s, 3H), 1.26 (d, *J* = 6.2 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  196.56, 154.91, 143.88, 138.66, 131.57, 129.75, 128.10, 122.65, 69.41, 53.97, 42.84, 31.90, 22.21, 21.18; HRMS (ESI) calcd for C<sub>20</sub>H<sub>27</sub>N<sub>5</sub>NaO<sub>2</sub>S<sub>2</sub> [M + Na]<sup>+</sup>: 456.1504, found: 456.1503.

**General procedure for the synthesis of compounds 93–95**—A mixture of **73** (0.70g, 2mmol), Et<sub>3</sub>N (0.76g, 2.2mmol) and CH<sub>3</sub>COCl (0.17g, 2.2mol) in CH<sub>2</sub>Cl<sub>2</sub> (10mL) was stirred at room temperature for 4.5 h. The disappearance of compounds **73** was monitored by TLC (silica gel, PE:Actone = 3:1). After the reaction, the solvent was washed with diluted hydrochloric acid, saturated NaHCO<sub>3</sub> (40 mL) and brine (40 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to give the crude product, which were recrystallized from acetone to provide compound **93** as white solid.

(1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 4-acetylpiperazine-1-carbodithioate (**93**): Yield 71.3%, white solid, mp. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.67 (s, 1H), 7.09–7.38 (m, 4H), 5.55 (s, 2H), 4.68 (s, 2H), 4.30 (brs, 2H), 4.00 (brs, 2H), 3.73 (t, *J* = 5.1 Hz, 2H), 3.60 (t, *J* = 5.1 Hz, 2H), 2.13 (s, 3H); HRMS (ESI) calcd for C<sub>17</sub>H<sub>20</sub>FN<sub>5</sub>NaOS<sub>2</sub> [M + Na]<sup>+</sup>: 416.0991, found: 416.0988.

**Cells and Cell Viability Assay:** The human gastric carcinoma cell lines MGC-803 and HGC-27 were supplied by the Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Human gastric carcinoma cell line SGC-7901 was purchased from Shanghai Bogoo Biotechnology Company. Human gastric epithelial mucosa cell line GES-1 was preserved in our institute. Cells were cultured in DMEM medium or RPMI-1640 medium. All media were supplemented with 10% FBS. Cells were cultured in an incubator with 5% CO<sub>2</sub> at 37 °C with medium changes every 2 days.

The cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay according to the manufacturer's instructions. This assay measures dehydrogenase enzyme activity in metabolically active tumor cells, as reflected by the conversion of MTT to formazan, which is soluble in tissue culture medium and is detected by absorbance (A) at 570 nm. The production of formazan is proportional to the number of living cells, with the intensity of the produced color serving as an indicator of cell viability. The data were analyzed with SPSS 20.

**Inhibitory Evaluation of the Synthesized Compounds Against LSD1, LSD2, MAO-A, MAO-B and Mechanism of Action Studies:** cDNA encoding LSD1(157-852AA) was obtained by RT-PCR and cloned into pET28b to have the constructed plasmid pET28b-LSD1, then the plasmid was transfected into BL21(DE). The recombinant was induced with 0.25mM IPTG at 20°C and purified as published, as well as pET28a-LSD2, which is a kindly gift from Dr. Chen.<sup>18, 69</sup> Then incubate the compounds with the recombinant and H3K4me2, after that, the fluorescence was measured at excitation wavelength 530 nm and emission wavelength 590 nm as reported in order to evaluate the inhibition rate of the candidate compound.<sup>73</sup> Inhibitory effect of the candidate compounds against MAO-A and MAO-B were evaluated with the commercialized kit.

The dilution assay was done as published.<sup>21</sup> Briefly, 2.5 µg of LSD1 recombinant were incubated with either 312.5 µM compound **26**, 600 µM tranlylcypromine, or DMSO. 1 h later, 1.25 µl aliquots were removed from all samples and diluted into HRP-assay solution containing substrate and coupling reagents to a final volume of 100 µl. This represents an 80-fold dilution of the inhibitor concentration, which is expected to yield the same inhibition rate for an irreversible inhibitor or significant difference for a reversible inhibitor.

For the dialysis experiment, compound **26** and LSD1 were incubate for 1 h at 37°C and then dialyzed against the hepes buffer at 4°C for 24 h. The buffer was changed every 12 h. Another two groups without inhibitor or with **2-PCPA** were served as negative or positive controls. Then, the activity of LSD1 in the dialyzed tube was measured to evaluate the reversibility of compound **26** inhibitory. Another dialysis experiment was performed, using FAD and LSD1 incubated with or without the inhibitor against hepes buffer at 4°C for 24 h. In the inhibitor treatment group, the concentrations of inhibitors on both sides of dialysis tube were the same. Then, the amount of FAD in the dialysis tubes was measured at 468nm with UV/VIS spectrophotometer.

For the competitive analysis of compound **26**, demethylase activity of LSD1 was assessed in the presence of different concentrations of the compound (0, 0.5, 2.5 and 12.5 µM) at a fixed concentration of FAD (2.5 µM) and peptide concentrations from 10 to 80 µM or fixed concentration of peptide (25 µM) and FAD concentrations from 0.6 to 9.0 µM. Assays were performed triplicate, and kinetic values were obtained using Lineweaver-Burk plots.<sup>59</sup>

**MST Experiment:** The LSD1 recombinant from Millipore was labeled with a red fluorescent dye using commercialized kit. The thermophoretic movement of the fluorescently labeled protein in complex with selected inhibitors was measured by monitoring the fluorescence distribution inside the capillary. The concentration of the labeled protein was kept constant at ~200 nM, while the concentration of the compound was varied. The samples were loaded into MST-grade glass capillaries. After a short incubation period, the MST analysis was performed using the Monolith NT.115.

**Docking and Molecular Dynamics Simulations:** The initial coordinates of LSD1 were obtained from Protein Data Bank (PDB) (PDB code: 2H94<sup>71</sup>). Two disordered loops with FASTA sequence EVKPPRDI and PSIPGAPQP were unresolved and thus built using



MODELLER 9.10.<sup>74</sup> The protein was then protonated at pH 7.0 using MCCE 2.2.<sup>75, 76</sup> The coordinates of the ligands **26** were obtained from their SMILES using OEChem TK (OpenEye Scientific Software). AM1-BCC charges were assigned as the partial charges of the ligands using Antechamber.<sup>77, 78</sup>

Multiple conformers of ligand were generated using OMEGA (OpenEye Scientific Software) and docked into the FAD-binding site of LSD1 using OEDocking (OpenEye Scientific Software), which uses an exhaustive search algorithm and Chemgauss3 scoring function.<sup>79</sup> One thousand binding poses were generated and clustered using a Root Mean Square Deviation (RMSD) metric to obtain ten most dissimilar clusters. Molecular dynamics (MD) simulations were subsequently performed on the centroid structures for each of the ten clusters using GROMACS 4.6.2.<sup>80</sup> The amber99sb-ildn and GAFF force fields were used for the protein and ligands, respectively.<sup>81, 82</sup> The protein-ligand complex was solvated in TIP3P water molecules. A 1500-step energy minimization and a 50 ps NVT equilibration simulation were performed at 300K before a 1ns production run was performed in NPT ensemble using a time step of 2 fs. Snapshots were saved every 1 ps for analysis. The ten ns production trajectories were concatenated for a Perron-Cluster Cluster Analysis (PCCA),<sup>83</sup> which generated ~20 distinct structure clusters.

**SiRNA Transfection:**  $6 \times 10^5$  cells were seeded in a 6 well plate and then incubated for 3 days in standard medium in the presence of 20–100 nM siRNA directed against LSD1 or control siRNA (Scrambled) complexed with Lipofectamine™ RNAiMAX Reagent according to the manufacturer's instructions.

**Migration and Invasion Assay:** For the wound healing assay, cells were plated to in a 24-well plate and the cell surface was scratched using a 10  $\mu$ L pipette tip. Then, cells were treated with compound **26** with different concentrations followed by 48 h incubation and photographed on an inverted microscope.

For invasion assays, cells were plated in transwell 24 Well plates coated with diluted Matrigel. In the upper chamber, medium was supplemented with 1% heat-inactivated FBS. In the lower chamber, 20% FBS was used as chemoattractant. Different concentrations of compound **26** were added in the chamber. After 48 h, medium was removed and the chambers were washed twice with PBS. Non-invading cells were removed from the upper surface of the membrane by scrubbing with cotton tipped swab, and the invading cells were fixed with methanol for 15 min. Then the chambers were stained with 0.1% crystal violet for 30 min. 6 fields for each chamber were photographed on an inverted microscope and invading cells were counted in each field.

For migration assays, cells were plated in transwell 24 Well plates without Matrigel. The medium, attractant, staining and cell counting method were the same as invasion assay.

**Immunoprecipitation and Western Blot:** Cross linked immunoprecipitation was performed with the commercialized kit from Thermo Fisher. Western Blot was performed with the total lysates by RIPA or histone purified with kit from Epigentek. Equal amounts of cell lysates were denatured, separated by SDS-PAGE and transferred to 0.2  $\mu$ m nitrocellulose membranes. After blocking with PBS containing 5% non-fat dry milk, the membranes were incubated overnight at 4°C with primary antibody, followed by incubation with a horseradish peroxidase-conjugated secondary antibody. The immunoblots were visualized by enhanced chemiluminescence kit from Thermo Fisher.

Antibodies used were against histone H3K4me (Selleck Chemicals #A1237), H3K4me2 (Epitomics #1347-1), H3K9me2 (Epitomics #1349-1), H3K4me3 (Bioss, bs-4715R), total



H3 (Sino Biological Inc #100005-MM01), LSD1 (Epitomics #5890-1), E-Cadherin (Epitomics #1702-1), Snai1 (Cell Signaling #3879), GAPDH (GoodHere #AB-M-M 001).

**Apoptotic Analysis:** Changes in cell morphology were analyzed by Hoechst-33258 staining. Cells were treated with compound **26** at the indicated concentrations for 48 h. Then the cells were fixed and stained with 5 $\mu$ g/mL Hoechst-33258. After staining, the cells were washed. Apoptotic cells were examined and identified according to the condensation and fragmentation of their nuclei by fluorescence microscopy.

The apoptosis was quantified by FACS with AnnexinV-FITC/PI Staining kit from Biovision. Ten thousand events for each sample were counted and analyzed by Accuri C6 flow cytometer. The early and late apoptotic cells were identified by the localization of Annexin V and PI.

**Xenograft Studies:** Xenografts models using human gastric cancer cell line (MGC-803) was established in BALB/C mice. Once the tumors reached 100 mm<sup>3</sup>, mice were divided into control groups (saline and hydroxypropyl- $\beta$ -Cyclodextrin) and treatment groups. The treatment groups received compound **26** (5, 10, 20 mg/kg prepared with hydroxypropyl- $\beta$ -Cyclodextrin) i.v. per day for a period of 21 days. Tumor volumes were measured at 3 days interval. After the 21st day, the mice were euthanized, and the tumors were isolated and weighed. Tumor size was determined by calliper measurements and the body weight was measured at 3 days interval to monitor drug toxicity.

**Statistical analyses:** Data were expressed as mean $\pm$ SD. The significance of the difference between different groups was determined with analysis of variance (ANOVA) and Student t test. Results were considered statistically significant at P<0.05, P<0.01 was considered highly significant.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## ABBREVIATIONS USED

<b>LSD1</b>	histone lysine specific demethylase 1
<b>LSD2</b>	histone lysine specific demethylase 2
<b>HKMT</b>	histone lysine methyltransferase
<b>HKDM</b>	histone demethylase
<b>DNMT</b>	DNA methyltransferase
<b>MAO</b>	monoamine oxidase
<b>HDAC</b>	histone deacetylase
<b>DSF</b>	disulfiram

<b>MRSA</b>	methicillin-resistant staphylococcus aureus
<b>FAD</b>	flavin adenine dinucleotide
<b>2-PCPA</b>	transylcypromine
<b>HPLC</b>	high performance liquid chromatography
<b>MST</b>	microscale thermophoresis
<b>HP-<math>\beta</math>-CD</b>	hydroxypropyl- $\beta$ -cyclodextrin
<b>FBS</b>	fetal bovine serum
<b>SD</b>	standard deviation

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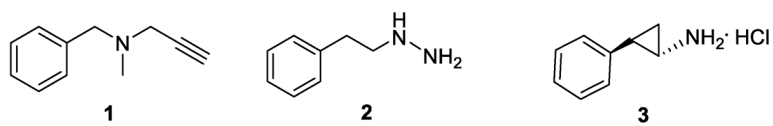
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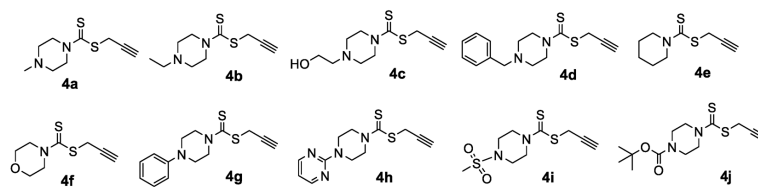
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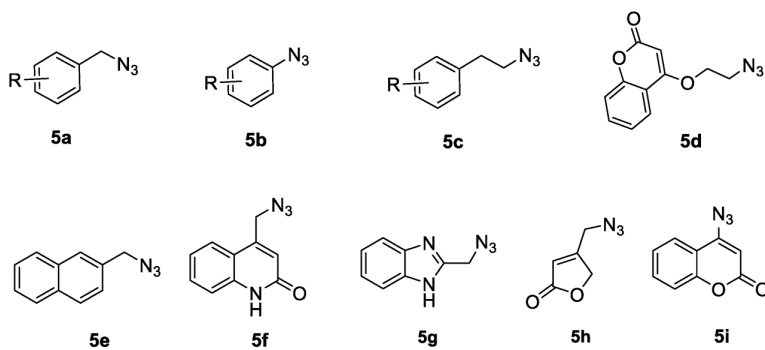
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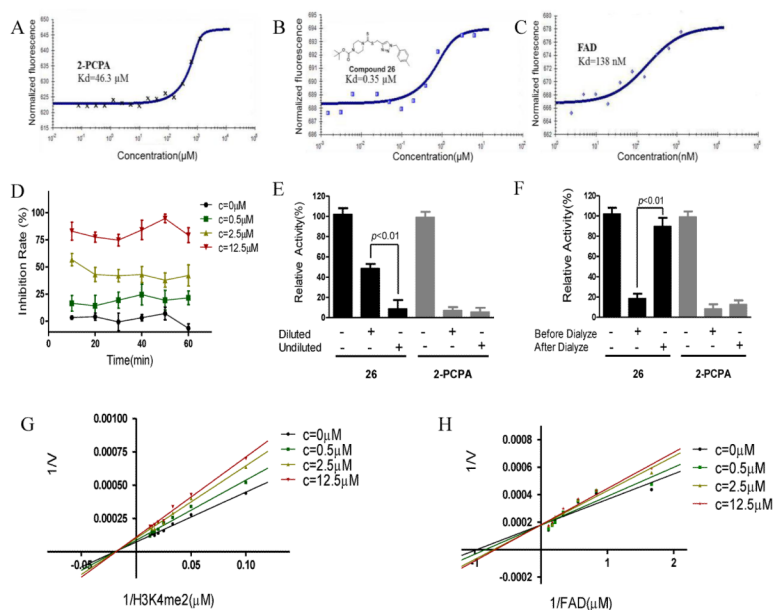
**Figure 1.**  
MAO inhibitors that inhibit LSD1



**Figure 2.**  
Alkyne intermediates 4a–4j used in the present study.

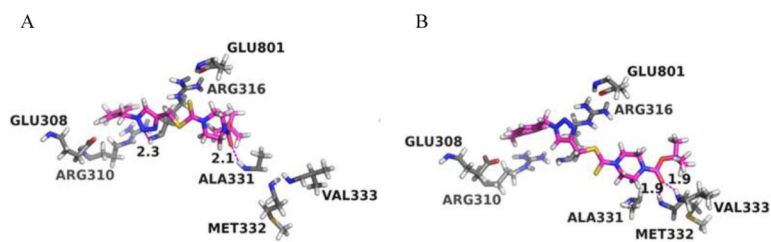


**Figure 3.**  
Azides 5a–5i used in the present study.



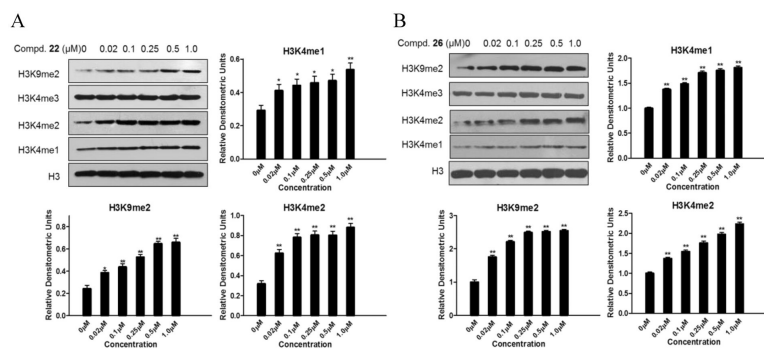
**Figure 4.**

Properties of compound **26**'s inhibitory effect to LSD1 activity *in vitro*. (A, B, C)  $K_d$  values for **2-PCPA** (A), compounds **26** (B) and FAD (C) were obtained by microscale thermophoresis (MST) experiment; (D) Time dependent assay of compound **26** against LSD1. The stable progress curves for the inactivation of LSD1 were obtained by indicated concentrations of compound **26** treatment; (E, F) The reversibility of compound **26** to LSD1 activity was determined by dilution assay (E) and dialysis experiment (F), **2-PCPA** was used a control; (G, H) Lineweaver–Burk plots demonstrate that compound **26** is non-competitive with the histone H3 substrate (G) and competitive with the LSD1 cofactor FAD (H). Data are mean $\pm$ SD.  $P < 0.01$  was considered statistically highly significant. All experiments were carried out at least three times.

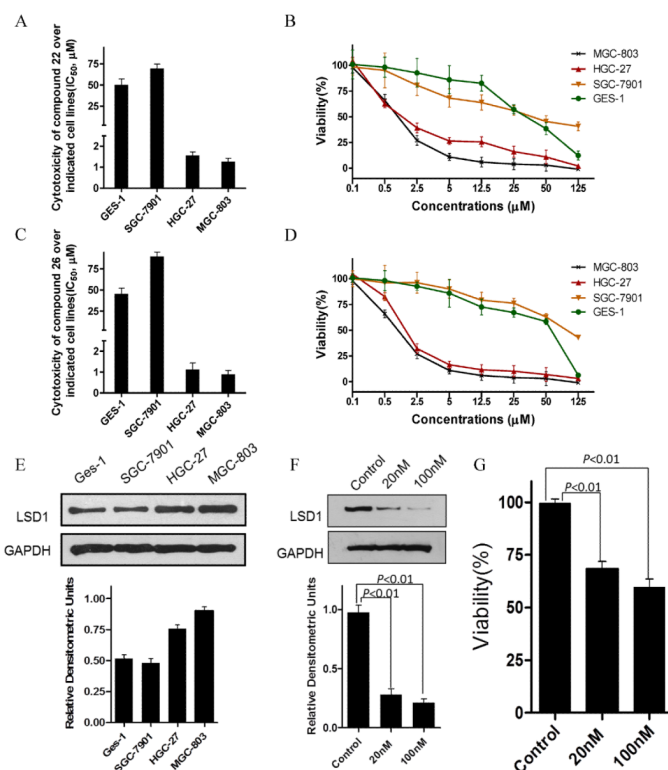


**Figure 5.** Stereo views of the putative binding modes of the ligands **26** in the FAD-binding site of LSD1. (A) **26**-Pose1; (B) **26**-Pose2. FAD-binding site residues are colored in grey while the ligand **26** is colored in magenta and green, respectively. Hydrogen bonds between the protein and ligands are shown in magenta dash lines with the distances (Å) between the donor hydrogen atom and the acceptor heavy atom labeled.

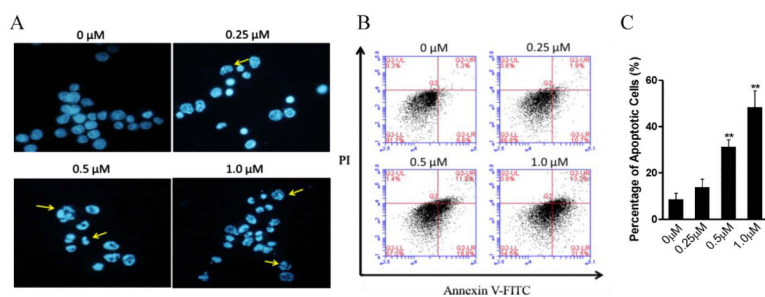




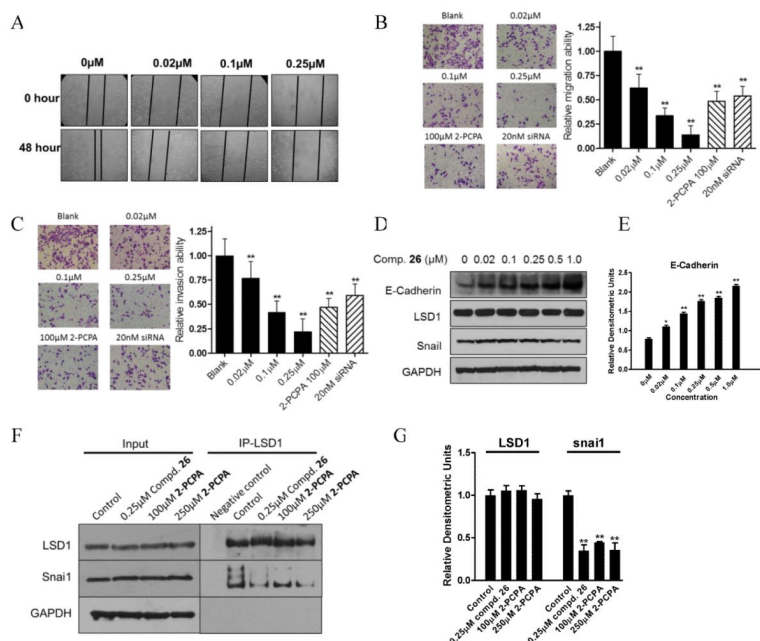
**Figure 6.** Histone methylation in MGC-803 cells after treatment by compound **22**(A) and **26**(B) for 48h. The expressions of H3K4me1, me2 and H3K9me2, as well as H3K4me3 were then determined by western blot. The total levels of histone 3 (H3) were used as loading control. Data are mean $\pm$ SD. \*P<0.05 was considered significant, \*\*P<0.01 was considered statistically highly significant. All experiments were carried out at least three times.

**Figure 7.**

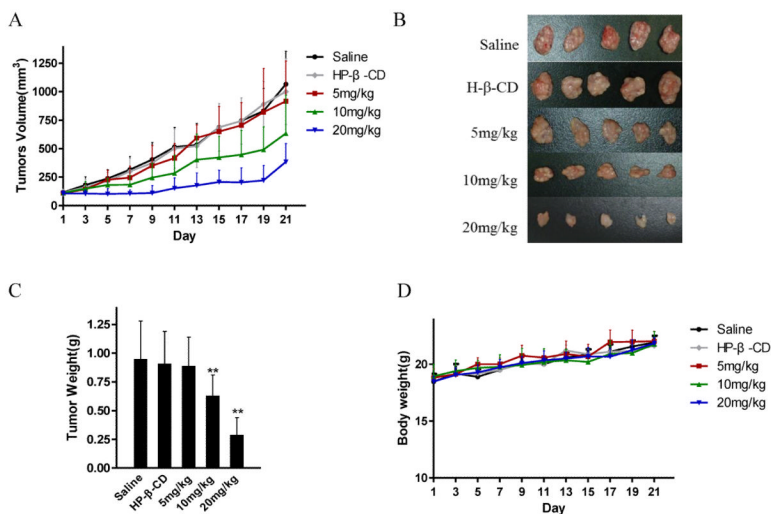
$IC_{50}$  and cell viability of compounds **22** (A and B), and compound **26** (C and D) treatment on three gastric cancer cell lines: MGC-803, HGC-27, SGC-7901 and normal gastric epithelial cell line: GES-1; (E) The expression levels of LSD1 on the four gastric cell lines indicated above; (F) 3 days after LSD1 siRNA transfection in MGC-803 cells, LSD1 protein levels were determined by western blot, GAPDH was used as the loading control. (G) Cell viability upon LSD1 siRNA treatment. Data are mean $\pm$ SD. \*\* $P < 0.01$  was considered statistically highly significant. All experiments were carried out at least three times.



**Figure 8.** Compound **26** induced apoptosis in MGC-803 cells. (A) Apoptosis analysis with Hoechst-33258 staining after 48h treatment of **26** in MGC-803 cells; (B and C) Quantitative analysis of apoptotic cells using AnnexinV-FITC/PI double staining and flow-cytometry calculation. \*\*P <0.01 was considered statistically highly significant. Data are mean±SD. All experiments were carried out at least three times.

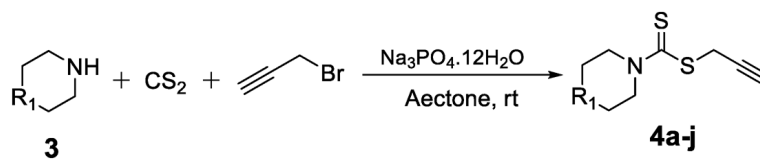


**Figure 9.** Effects of compound **26** on cell migration, invasion in MGC-803 cells. (A) Wound healing assay; (B and C) Migration and invasion ability assay. Treatment of both compound **26** and LSD1 siRNA resulted in the depressed migration (B) and invasion (C) ability in MGC-803 cells, **2-PCPA** was used as the positive control; (D and E) The expressions of E-Cadherin, snail and LSD1 after 48h treatment of compound **26**; (F and G) Immunoprecipitation and densitometry quantitation of LSD1 and snail without (control) or with compound **26** and 100, 250 μM **2-PCPA** treatment in MGC-803 cells; input represents cell lysate controls. GAPDH was used as loading control. \* $P < 0.05$  was considered statistically significant, \*\* $P < 0.01$  was considered statistically highly significant. Data are mean  $\pm$  SD. All experiments were carried out at least three times.



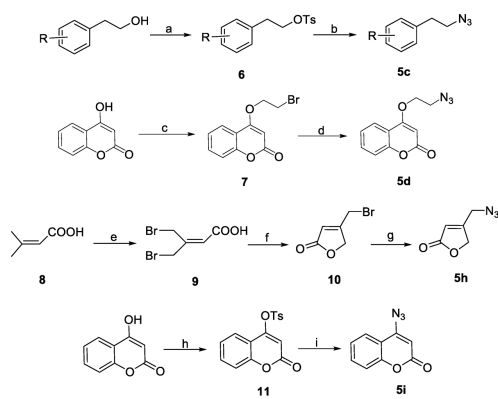
**Figure 10.**

*In vivo* antitumor effects of compound **26** in MGC-803 bearing nude mice. MGC-803 cells were transplanted subcutaneously to the BALB-C nude mice and subjected to saline, hydroxypropyl-β-cyclodextrin (HP-β-CD) and compound **26** (5, 10, 20 mg/kg) for 21 days, respectively, and were then analyzed for tumor volume (A); (B) Represented tumors with the indicated treatment; (C) Tumor weight with the indicated treatment; (D) Body weight with the indicated treatment. \*P < 0.05 was considered statistically significant, \*\*P < 0.01 was considered statistically highly significant. Data are mean ± SD.



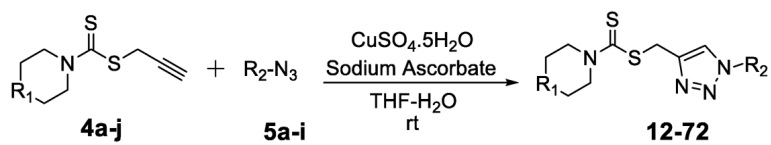
**Scheme 1.**  
Synthesis of alkyne intermediates.



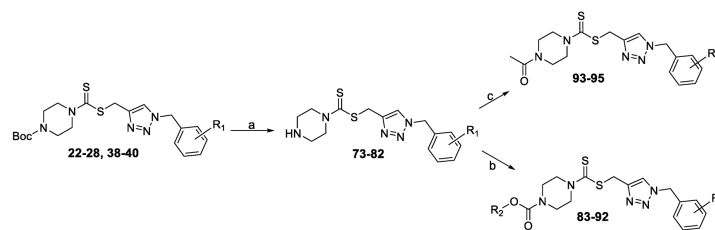


<sup>a</sup>Reagents and conditions: (a) TsCl, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, rt; (b) NaN<sub>3</sub>, CH<sub>3</sub>CN, reflux; (c) 1,2-dibromoethane, K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN, reflux; (d) NaN<sub>3</sub>, Acetone-H<sub>2</sub>O (4:1), reflux; (e) NBS, Ph(CO)<sub>2</sub>O<sub>2</sub>, CCl<sub>4</sub>, reflux; (f) 5% aq NaOH, rt, 12 h; (g) NaN<sub>3</sub>, Acetone-H<sub>2</sub>O (4:1), rt; (h) TsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt; (i) NaN<sub>3</sub>, DMF, 80°C.

**Scheme 2.**  
Synthesis of the azides 5c, 5d, 5h and 5i<sup>a</sup>

**Scheme 3.**

Synthesis of 1, 2, 3-triazole-dithiocarbamate hybrids 12–72 through click chemistry.



<sup>a</sup>Reagents and conditions: (a)  $\text{CF}_3\text{COOH}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ -rt; (b)  $\text{R}_2\text{OCOCl}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{CH}_2\text{Cl}_2$ , rt;

(c)  $\text{CH}_3\text{COCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , rt.

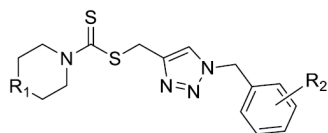
**Scheme 4.**

Synthesis of 1, 2, 3-triazole-dithiocarbamate hybrids 73–96<sup>a</sup>.

Table 1

Structures of compounds **12–40** and their inhibition rates (IC<sub>50</sub>) to the purified LSD1 recombinant *in vitro*.

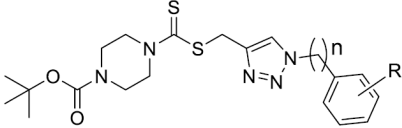
Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> (μM)	Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> (μM)
12	CH <sub>2</sub>	<i>o</i> -F	>125	27		<i>p</i> -OCH <sub>3</sub>	18.4±2.5
13	CH <sub>2</sub>	<i>p</i> -F	>125	28		<i>m</i> -CH <sub>3</sub>	43.3±5.9
14	CH <sub>2</sub>	<i>p</i> -CH <sub>3</sub>	>125	29		<i>o</i> -CH <sub>3</sub>	>125
15	CH <sub>2</sub>	H	>125	30		<i>p</i> -CF <sub>3</sub>	>125
16	CH <sub>2</sub>	<i>p</i> -Cl	>125	31		<i>o</i> -CF <sub>3</sub>	>125
17	0	H	>125	32		<i>o</i> -Br	27.5±3.7
18	0	<i>o</i> -F	>125	33			70.7±1.2
19	0	<i>p</i> -F	>125	34		<i>o</i> -OH	69.2±3.6
20	0	<i>p</i> -CH <sub>3</sub>	>125	35		<i>p</i> -NO <sub>2</sub>	>125



Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> ( $\mu$ M)	Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> ( $\mu$ M)
21	0	<i>p</i> -Cl	>125	36		<i>m</i> -OH	77.1 $\pm$ 3.1
<u>22</u>		<i>o</i> -F	<u>8.0<math>\pm</math>1.0</u>	37		<i>o, p</i> -diOH	81.3 $\pm$ 2.0
23		<i>p</i> -F	21.6 $\pm$ 2.3	38		<i>m, p</i> -diCl	88.9 $\pm$ 3.2
24		<i>o</i> -Cl	11.5 $\pm$ 2.1	39		<i>o, o</i> -diF	51.2 $\pm$ 4.3
25		<i>p</i> -Cl	56.5 $\pm$ 2.8	40		<i>m, p, m</i> -triOCH <sub>3</sub>	19.0 $\pm$ 2.5
<u>26</u>		<i>p</i> -CH <sub>3</sub>	<u>2.1<math>\pm</math>0.7</u>	2-PCPA	-	-	27.8 $\pm$ 2.6

**Table 2**

The effect of linker length on LSD1 inhibition.



Comp.	n	R	IC <sub>50</sub> ( $\mu$ M)
41	0	<i>p</i> -CH <sub>3</sub>	>125
42	0	<i>p</i> -OCH <sub>3</sub>	>125
43	0	<i>o</i> -F	>125
44	0	<i>m</i> -CF <sub>3</sub>	>125
45	2	<i>p</i> -CH <sub>3</sub>	>125
46	2	<i>p</i> -F	>125

**Table 3**

The Effect of different aromatic rings on LSD1 inhibition.

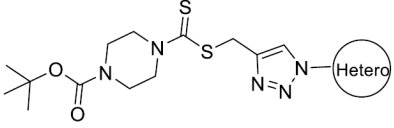
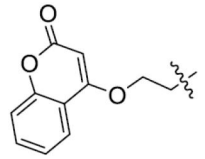
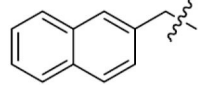
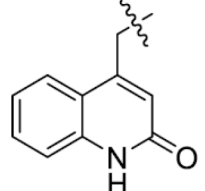
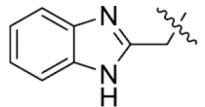
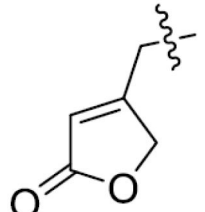
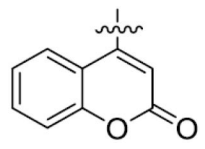
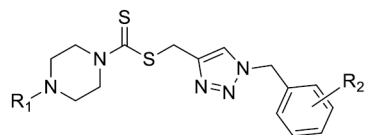
Comp.	Hetero	IC <sub>50</sub> ( $\mu$ M)
		
47		>125
48		>125
49		>125
50		>125
51		>125
52		>125

Table 4

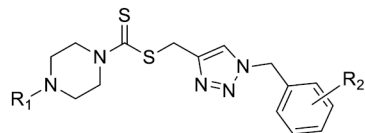
The effect of different substituents at the N-atom on LSD1 inhibition.

Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> (μM)	Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> (μM)
53	CH <sub>3</sub>	<i>o</i> -F	>125	68		<i>o</i> -F	39.8±1.1
54	CH <sub>3</sub>	<i>p</i> -CH <sub>3</sub>	>125	69		<i>p</i> -F	43.9±2.5
55	CH <sub>3</sub>	<i>p</i> -F	>125	70		<i>p</i> -CH <sub>3</sub>	35.7±2.0
56	CH <sub>3</sub>	<i>o</i> -Cl	>125	71		<i>o</i> -F	45.7±1.9
57		<i>p</i> -CH <sub>3</sub>	>125	72		<i>p</i> -F	55.1±3.3





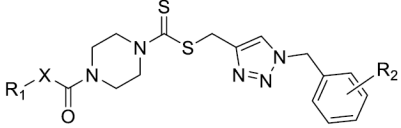
Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> (μM)	Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> (μM)
58		<i>o</i> -F	>125	73	H	<i>o</i> -F	17.3±1.7
59		<i>p</i> -CH <sub>3</sub>	>125	74	H	<i>p</i> -F	69.3±1.8
60		<i>o</i> -F	>125	75	H	<i>o</i> -Cl	>125
61		<i>p</i> -CH <sub>3</sub>	83.4±2.1	76	H	<i>p</i> -Cl	>125
62		<i>o</i> -F	100.1±9.3	77	H	<i>p</i> -CH <sub>3</sub>	28.9±2.0
63		<i>p</i> -F	>125	78	H	<i>p</i> -OCH <sub>3</sub>	19.5±1.9
64		<i>p</i> -CH <sub>3</sub>	40.3±2.1	79	H	<i>p</i> -CF <sub>3</sub>	87.4±2.3
65		<i>o</i> -F	>125	80	H	<i>o, o</i> -diF	>125

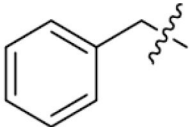
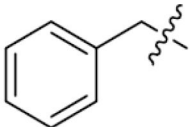
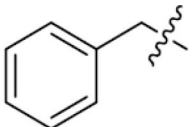
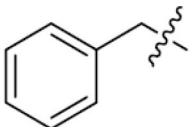
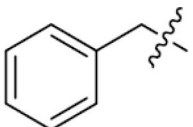
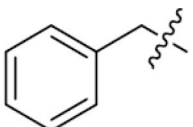
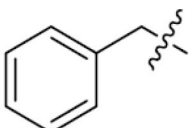


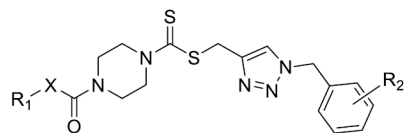
Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> ( $\mu$ M)	Comp.	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> ( $\mu$ M)
66		<i>p</i> -F	>125	81	H	<i>m, p</i> -diCl	>125
67		<i>p</i> -CH <sub>3</sub>	27.6 $\pm$ 1.2	82	H	<i>m, p, m</i> -triOCH <sub>3</sub>	>125

**Table 5**

The Effect of the bulk of carbamate moiety on LSD1 inhibition.



Comp.	R <sub>1</sub>	R <sub>2</sub>	X	IC <sub>50</sub> ( $\mu$ M)
83		<i>o</i> -F	0	32.8 $\pm$ 2.0
84		<i>p</i> -F	0	47.6 $\pm$ 4.3
85		<i>p</i> -Cl	0	53.2 $\pm$ 2.9
86		<i>p</i> -Cl	0	>125
87		<i>p</i> -CH <sub>3</sub>	0	22.4 $\pm$ 1.0
88		<i>p</i> -OCH <sub>3</sub>	0	35.1 $\pm$ 2.1
89		<i>m, p</i> -diCl	0	29.5 $\pm$ 1.3



Comp.	R <sub>1</sub>	R <sub>2</sub>	X	IC <sub>50</sub> (μM)
90		<i>m,p,m</i> -triOCH <sub>3</sub>	0	43.5±4.1
91		<i>p</i> -CH <sub>3</sub>	0	17.0±1.2
92		<i>p</i> -CH <sub>3</sub>	0	15.0±0.7
93	H	<i>o</i> -F	CH <sub>2</sub>	30.2±1.9
94	H	<i>p</i> -F	CH <sub>2</sub>	47.1±2.0
95	H	<i>p</i> -CH <sub>3</sub>	CH <sub>2</sub>	25.3±2.2

**Table 6**

*In vitro* inhibitory activities of compound **22**, **26** to LSD1 and its homologues: LSD2, MAO-A, and MAO-B. **2-PCPA** was used as control.

Compd.	IC <sub>50</sub> ( $\mu$ M)			
	LSD1	LSD2	MAO-A	MAO-B
<b>22</b>	11.5 $\pm$ 2.1	49.1 $\pm$ 7.3	>1250	790.7 $\pm$ 10.4
<b>26</b>	2.1 $\pm$ 1.1	36.6 $\pm$ 4.5	>1250	>1250
<b>2-PCPA</b>	28.7 $\pm$ 3.5	34.9 $\pm$ 5.3	10.6 $\pm$ 1.0	5.9 $\pm$ 0.8