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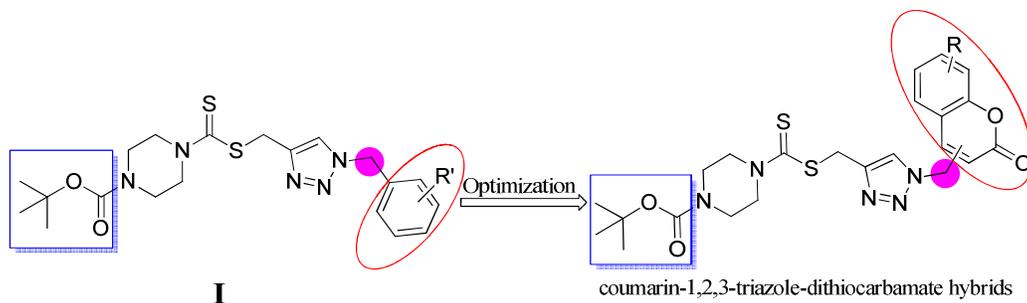
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**Table of Content:**

Design the novel coumarin-1,2,3-triazole-dithiocarbamate hybrids as potent LSD1 inhibitors by introducing the coumarin scaffold.



# Synthesis and biological evaluation of coumarin-1, 2, 3-triazole-dithiocarbamate hybrids as potent LSD1 inhibitors

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**Abstract:** Two series of coumarin-1,2,3-triazole-dithiocarbamate hybrids were designed, synthesized and evaluated for their inhibitory activity towards lysine specific demethylase 1 (LSD1). Compounds **8a**, **8d-8f**, **8i-8l** presented potent activity against lysine specific demethylase 1. Among them, compound **8k** showed potent and reversible inhibition against lysine specific demethylase 1 with an IC<sub>50</sub> value of 0.39 μM, which was 74-fold more potent than that of tranylcypromine (2-PCPA). Besides, compound **8k** displayed excellent selectivity against lysine specific demethylase 1 without inhibition against monoamine oxidases (MAOs) A and B. Further investigation revealed that compound **8k** was active in both recombinant and cell level by upregulating the expression of H3K4me1, H3K4me2 and H3K9me2.

**Keywords:** Coumarin; 1, 2, 3-Triazole; Dithiocarbamate; lysine specific demethylase 1

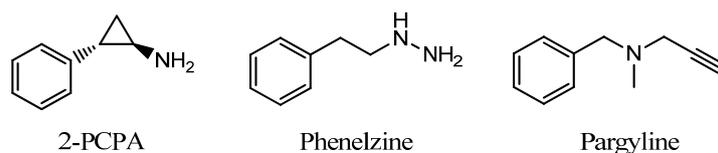
## Introduction

Histone modifications, including methylation, acetylation, phosphorylation and hydroxylation, play an important role in the epigenetic control of gene expression. Among these modifications, histone lysine methylation is reversibly regulated by histone lysine methyltransferases (HKMTs) and demethylases (HDMs). Lysine specific demethylase 1 (LSD1), the first characterized histone lysine demethylase discovered in 2004, removes the methyl groups from mono-, di-methylated Lys4 and Lys9 of histone H3 (H3K4, H3K9) through flavin adenine dinucleotide (FAD) dependent enzymatic oxidation.<sup>[1]</sup> LSD1 could also demethylate p53,<sup>[2]</sup> DNA methyltransferase 1,<sup>[3]</sup> E2F

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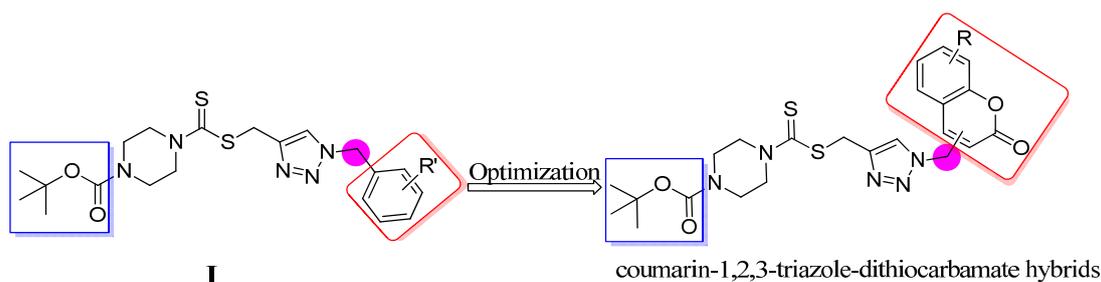
<sup>†</sup> These authors contributed equally.

transcription factor 1 (E2F1),<sup>[4]</sup> and regulate their cellular functions. Besides, downregulation of LSD1 expression or inhibition of its activity can inhibit cancer progression.<sup>[5-7]</sup> Hence, LSD1 has been considered as an ideal target for the treatment of cancer. LSD1 is a member of monoamine oxidase (MAO) family, which shows homology with monoamine oxidases (MAOs) A and B (17.6% identity). As reported, MAO inhibitors (Figure 1), such as tranlycypromine (2-PCPA), phenelzine and pargyline, have been evaluated as inhibitors of LSD1.<sup>[8]</sup> However, more novel LSD1 inhibitors have rarely been studied.<sup>[9-13]</sup>



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

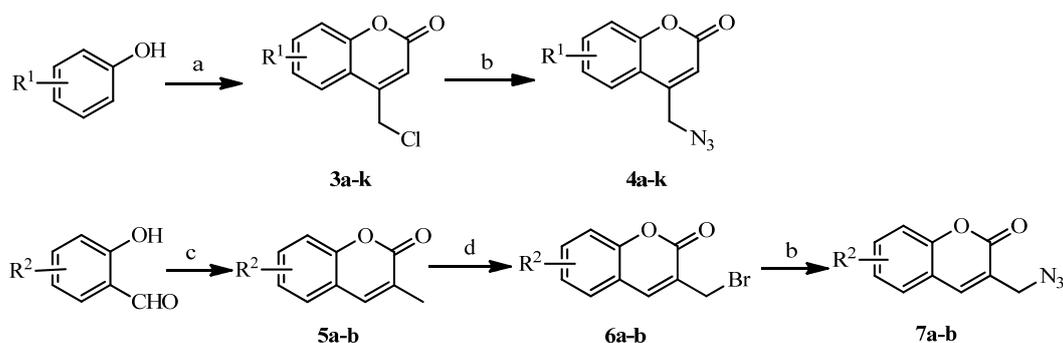
Coumarin-containing molecules have attracted great interests because of their diverse biological activities, such as anticancer,<sup>[14]</sup> antioxidant, anti-inflammatory,<sup>[15]</sup> antimicrobial,<sup>[16]</sup> and enzymatic inhibition.<sup>[17, 18]</sup> Particularly, some coumarins were described as monoamine oxidase inhibitors.<sup>[17]</sup> In our previous work, we reported the synthesis and biological activities of a series of 1,2,3-triazole-dithiocarbamate hybrids (Figure 2, I). Several compounds showed excellent broad spectrum anticancer activity and good anti-LSD1 activities.<sup>[19-21]</sup> The preliminary Structure-activity relationship (SAR) studies revealed that the tert-butyloxycarbonyl group attached to the piperazine ring and only one carbon length between triazole ring and the phenyl ring were critical for their inhibitory activity. So in this study, these two biologically important groups are retained. Another intriguing finding was that substituents on the phenyl ring displayed marked impact on its anti-LSD1 activity. In continuation with our efforts toward the discovery of novel anti-LSD1 agents,<sup>[21]</sup> and inspired by the significant activities of coumarins against MAO,<sup>[17]</sup> we herein design the novel coumarin-1,2,3-triazole-dithiocarbamate hybrids by introducing the coumarin scaffold and further evaluate their anti-LSD1 activity.



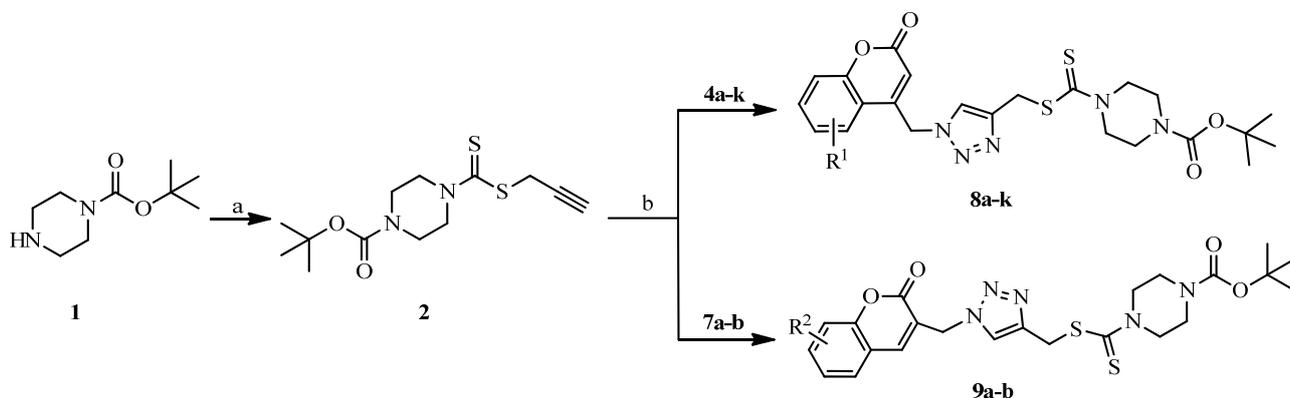
**Figure 2.** Designed structures of coumarin-1,2,3-triazole-dithiocarbamate hybrids

## Results and discussion

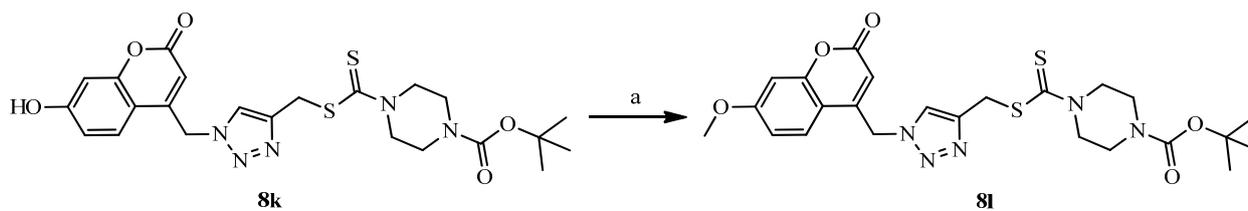
The synthetic routes for coumarin-1, 2, 3-triazole-dithiocarbamate hybrids **8a-k** and **9a-b** are outlined in Scheme 1-3. The key intermediate **2** was efficiently prepared following our previous described method.<sup>[19]</sup> Compounds **4a-k** were obtained by reaction of sodium azide with **3a-k** that were reached from phenols and ethyl 4-chloroacetoacetate by using Pechman condensation conditions. Condensation of substituted salicylaldehyde with propanoic anhydride in refluxing propionic anhydride gave compounds **5a-b** in modest yield.<sup>[22]</sup> Compounds **7a-b** were synthesized from compounds **6a-b** employing the similar conditions for the synthesis of Compounds **4a-k**. Compounds **6a-b** were generated from the AIBN mediated bromination of **5a** and **5b** with NBS.<sup>[23]</sup> Finally, compounds **8a-k** and **9a-b** were obtained from alkyne **2** and corresponding azides through the Huisgen 1, 3-dipolar cycloaddition. Compound **8l** was synthesized through methylation of **8k** in the presence of  $K_2CO_3$ .



**Scheme 1.** Synthesis of the azides (**4a-k** and **7a-b**). Reagents and conditions: (a) Ethyl 4-chloroacetoacetate, 70%  $H_2SO_4$ , 0 °C; (b)  $NaN_3$ ,  $CH_3CN$  or acetone/ $H_2O$ ; (c)  $CH_3CH_2COONa$ ,  $(CH_3CH_2CO)_2O$ ,  $Et_3N$ , reflux; (d) NBS, AIBN,  $CCl_4$ , reflux.

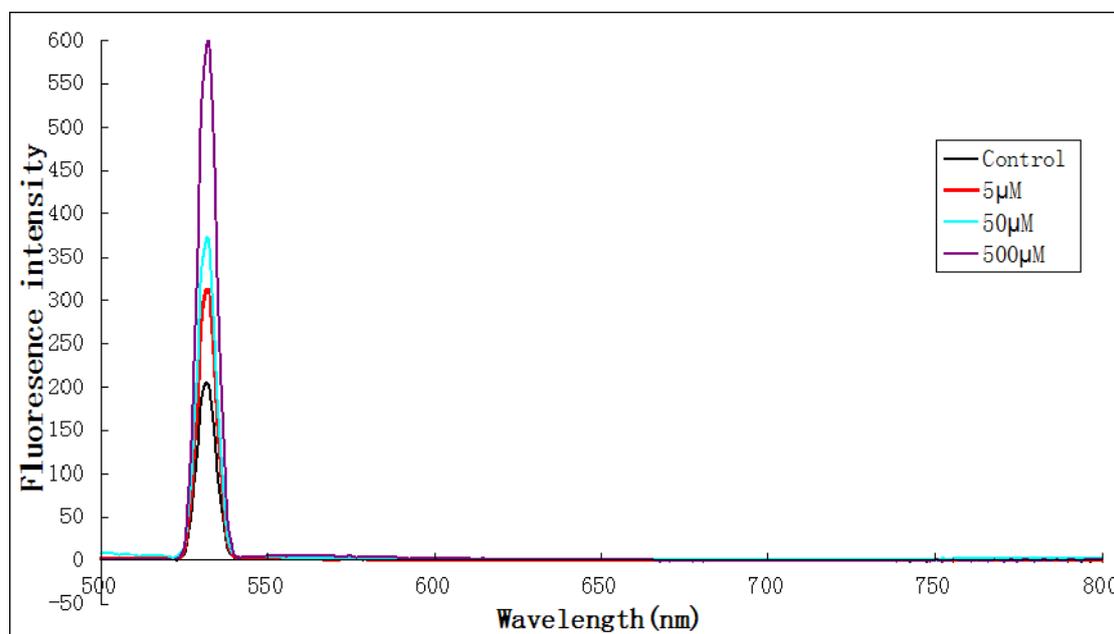


**Scheme 2.** Synthesis of the coumarin-1, 2, 3-triazole-dithiocarbamate hybrids (**8a-k** and **9a-b**). Reagents and conditions: (a)  $CS_2$ ,  $Na_3PO_4 \cdot 12H_2O$ , propargyl bromide, acetone, rt; (b)  $CuSO_4 \cdot 5H_2O$ , sodium ascorbate, THF- $H_2O$  (1/1), rt.



**Scheme 3.** Synthesis of the coumarin-1, 2, 3-triazole-dithiocarbamate hybrid (**8l**). Reagents and conditions: (a) DMF, K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>I, 80 °C.

In order to determine the inhibitory activity of the synthesized compounds against LSD1, we generated LSD1 recombinant expressing vector containing human LSD1 cDNA. The expression of recombinant LSD1 was then induced in *Escherichia Coli* (*E.Coli*) and purified according to the reported method.<sup>[24]</sup> The demethylase activity of the recombinant LSD1 was further determined by a fluorescence-based method, using synthesized H3K4me2 as a substrate.<sup>[25]</sup> The emission wavelength for LSD1 inhibitor screening was 590 nm and the excitation wavelength was 530 nm. To eliminate the possible artifacts caused by the fluorescence nature of these compounds, we did an experiment about the fluorescence scanning of compound **8k**. As shown in Figure 3, there was no fluorescence absorption at around 590 nm (the detection wavelength), indicating the fluorescence nature of compound **8k** had no effect towards its fluorescence absorption at around 590 nm.



**Figure 3.** Fluorescence scanning of compound **8k** at 530 nm excitation wavelength

In our previous work,<sup>[21]</sup> the preliminary SAR studies revealed that one carbon length between

triazole ring and the phenyl ring was optimal. So the coumarin hybrids reported were inactive against LSD1, and we retained the biologically important group in this study. All the compounds synthesized were examined for their *in vitro* inhibitory effect on LSD1 activity and the results were summarized in Table 1. 2-PCPA was chosen as a positive control. As shown in Table 1, most of the synthesized compounds exhibited moderate to excellent inhibitory activity towards LSD1 with  $IC_{50}$  values ranging from 0.39 to 102.56  $\mu\text{M}$ . Among them, compound **8k** showed the most potent activity against LSD1 ( $IC_{50} = 0.39 \mu\text{M}$ ), which was 74 -fold more potent than 2-PCPA. Moreover, compounds **8a**, **8d-8f** and **8i-8l** were also more potent than 2-PCPA. The substituents on coumarins had a profound effect on LSD1 inhibitory activity. Specifically, incorporation of chloro and methyl group into 7-position of coumarin nucleus (**8a** and **8f**) showed improved inhibition against LSD1 with the  $IC_{50}$  values in the nanomole range. By contrast, compounds **8b** and **8g** with chloro atom and methyl on the 6-position of coumarin represented no inhibitory activity. Compared with **8h**, compound **8i**, with 5, 7-dihydroxy group represented excellent inhibitory activity towards LSD1 ( $IC_{50} = 3.00 \mu\text{M}$ ). Besides, compounds **8d** and **8k** having a triazolyl group attached to the 4-position of coumarin nucleus had great inhibitory activity towards LSD1 with the  $IC_{50}$  values of 10.33 and 0.39  $\mu\text{M}$ , respectively. While for compounds **9a** and **9b**, the activity was totally lost.

**Table 1.** Preliminary *in vitro* inhibitory activities of compounds **8a-l**, **9a-b** ( $IC_{50}$ ) toward LSD1.

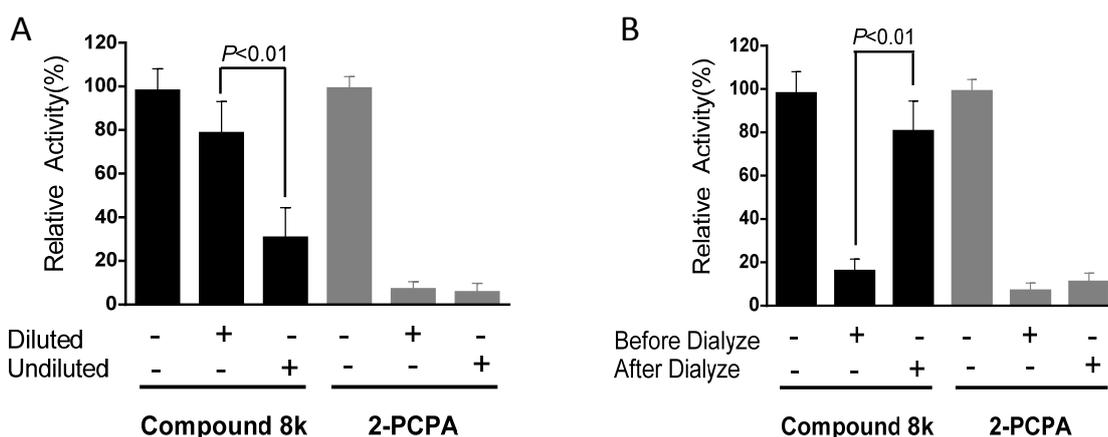
Comp.	R <sup>1</sup>	R <sup>2</sup>	LSD1( $\mu\text{M}$ )	Comp.	R <sup>1</sup>	R <sup>2</sup>	LSD1( $\mu\text{M}$ )
<b>8a</b>	7-Cl	-	0.67±0.29	<b>8i</b>	5,7-diOH	-	3.00±1.32
<b>8b</b>	6-Cl	-	>125	<b>8j</b>	7,8-diOH	-	0.83±0.23
<b>8c</b>	7-F	-	84.2±2.47	<b>8k</b>	7-OH	-	0.39±0.15
<b>8d</b>	H	-	10.33±1.09	<b>8l</b>	7-OCH <sub>3</sub>	-	0.81±0.40
<b>8e</b>	7-NH <sub>2</sub>	-	0.53±0.11	<b>9a</b>	-	H	>125
<b>8f</b>	7-CH <sub>3</sub>	-	0.71±0.31	<b>9b</b>	-	7-OH	102.56±5.23
<b>8g</b>	6-CH <sub>3</sub>	-	>125	2-PCPA	-	-	28.73±1.21
<b>8h</b>	5-CH <sub>3</sub> ,7-OH	-	>125				

As LSD1 belongs to the monoamine oxidase family, in order to evaluate the selectivity of the inhibitors, inhibitory effects of compound **8k** to MAO-A and MAO-B were investigated, and 2-PCPA was chosen as a positive control. As shown in Table 2, compound **8k** had no inhibitory

effects on MAO-A and MAO-B. While compound **8k** showed potent inhibition with the  $IC_{50}$  value of  $0.39 \pm 0.15 \mu\text{M}$  (74-fold more potent than that of 2-PCPA). The findings indicated the high selectivity of compound **8k** on LSD1 *in vitro*. Besides, reversibility was also evaluated with dilution assay and dialysis experiment.<sup>[21]</sup> As shown in Figure 4, the results indicated the reversibility of compound **8k**, compared to 2-PCPA.

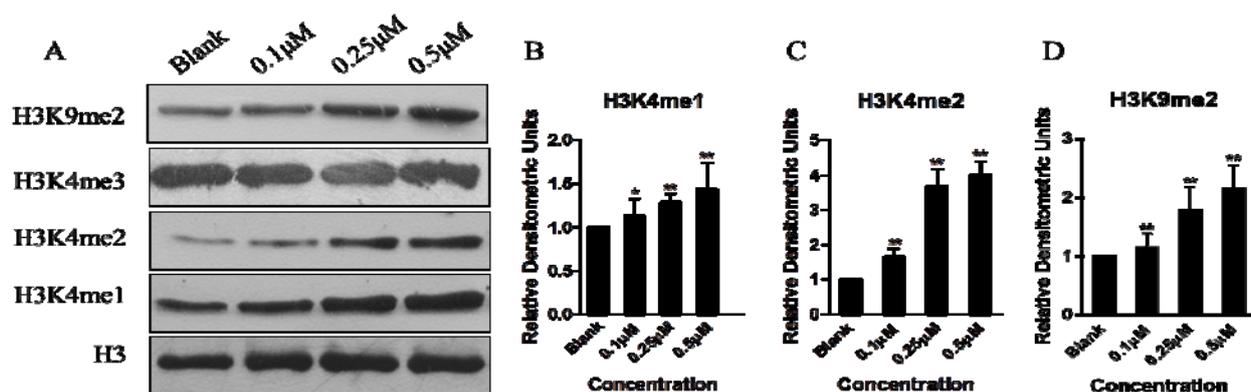
**Table 2.** *In vitro* inhibitory activities of compound **8k** to LSD1 and MAO-A, and MAO-B

Compounds	$IC_{50}(\mu\text{M})$		
	LSD1	MAO-A	MAO-B
<b>8k</b>	$0.39 \pm 0.15$	$>1250$	$>1250$
2-PCPA	$28.73 \pm 1.21$	$10.63 \pm 1.02$	$5.9 \pm 0.85$



**Figure 4.** The reversibility of compound **8k** to LSD1 activity was determined by dilution assay (A) and dialysis experiment (B).

To further evaluate the cell level LSD1 inhibitory effect, human gastric cancer cell line MGC-803 histone was extracted and subjected to western blot analysis with the treatment of compound **8k**. As shown in Figure 5 (A, B, C, D), elevated expression of H3K4me1/2 and H3K9me2 could be found, which suggested the activity of LSD1 may be inhibited by compound **8k**. But no obvious change of H3K4me3 can be observed, which illustrated the selectivity of compound **8k**. Meanwhile, the total amount of histone 3 was not changed. The results strongly suggested that the novel coumarin-1,2,3-triazole-dithiocarbamate hybrid LSD1 inhibitor was not only active in recombinant level, but also active in cell level.



**Figure 5.** Histone methylation in MGC-803 cells after treatment with compound **8k** for 48h. (A) Expression level of H3K4me1, H3K4me2, H3K4me3, and H3K9me2 were determined by western blot; (B) Densitometry quantitation of H3K4me1 with indicated treatment; (C) Densitometry quantitation of H3K4me2 with indicated treatment. (D) Densitometry quantitation of H3K9me2 with indicated treatment. Total amount of histone 3 (H3) were used as loading control.

## Conclusion

In conclusion, we report the synthesis and *in vitro* inhibitory activity towards LSD1 of coumarin-1,2,3-triazole-dithiocarbamate hybrids. The substituents on coumarins had a profound influence on LSD1 inhibitory activity. Compounds **9a** and **9b** with the triazolyl group connected to 3-position of coumarin nucleus lost their inhibitory activity towards LSD1. Most of the mono-substituted coumarins at the 7-position had excellent inhibitory activity. Among them, compound **8k** ( $IC_{50} = 0.39 \mu\text{M}$ ) was 74-fold more potent than 2-PCPA and more potent than our previously published compounds.

## Acknowledgments

We are grateful for the financial support from the National Natural Science Foundation of China (Project No. 81172937, U1204206), China Postdoctoral Science Foundation funded project (Project No. 20100480857, 201104402) and New Teachers' Fund for Doctor Stations, Ministry of Education (Project No. 20114101120013).

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