MedChemComm

CONCISE ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Med. Chem. Commun.*, 2014, **5**, 650

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

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Received 23rd January 2014
Accepted 1st March 2014

DOI: 10.1039/c4md00031e

www.rsc.org/medchemcomm

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_{50} 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 at both recombinant and cell levels by upregulating the expression of H3K4me1, H3K4me2 and H3K9me2.

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 (HLMTs) 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 and H3K9) through flavin adenine dinucleotide (FAD) dependent enzymatic oxidation.[1] LSD1 could also demethylate p53,[2] DNA methyltransferase 1,[3] E2F transcription factor 1 (E2F1),[4] and regulate their cellular functions. Besides, the downregulation of LSD1 expression or inhibition of its activity can inhibit cancer progression.[5–7] Hence LSD1 has been considered as an ideal target for the treatment of cancer. LSD1 is a member of the monoamine oxidase (MAO) family, which shows homology with monoamine oxidases (MAOs) A and B (17.6% identity). As reported, MAO inhibitors (Fig. 1), such as tranylcypromine (2-PCPA), phenelzine and pargyline, have been evaluated as inhibitors of LSD1.[8] However, more novel LSD1 inhibitors have rarely been studied.[9–13]

Coumarin-containing molecules have attracted great interest because of their diverse biological activities, such as anticancer,[14] antioxidant, anti-inflammatory,[15] antimicrobial,[16] and enzymatic inhibition.[17,18] Particularly, some coumarins were described as monoamine oxidase inhibitors.[17] In our previous work, we reported the synthesis and biological activities of a series of 1,2,3-triazole–dithiocarbamate hybrids (Fig. 2, **I**). Several compounds showed an excellent broad spectrum of anticancer activity and good anti-LSD1 activities.[19–21] The preliminary structure–activity relationship (SAR) studies revealed that the *tert*-butyloxycarbonyl group attached to the piperazine ring and only one carbon length between the 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,[21] and inspired by the significant activities of coumarins against MAO,[17] we herein design novel coumarin–1,2,3-triazole–dithiocarbamate hybrids by introducing a coumarin scaffold and further evaluate their anti-LSD1 activity.

Results and discussion

The synthetic routes to coumarin–1,2,3-triazole–dithiocarbamate hybrids **8a–l** and **9a–b** are outlined in Schemes 1–3. Key

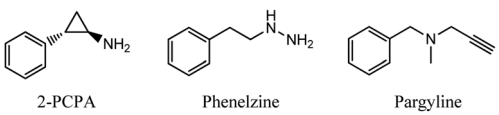

Fig. 1 MAO inhibitors that inhibit LSD1.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c4md00031e

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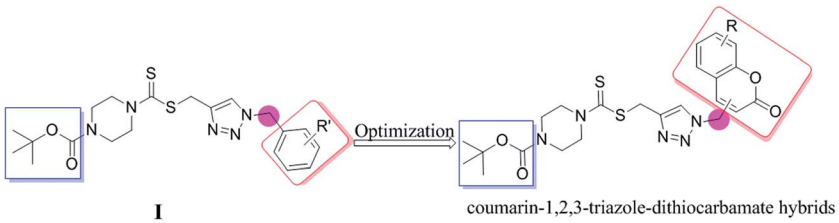
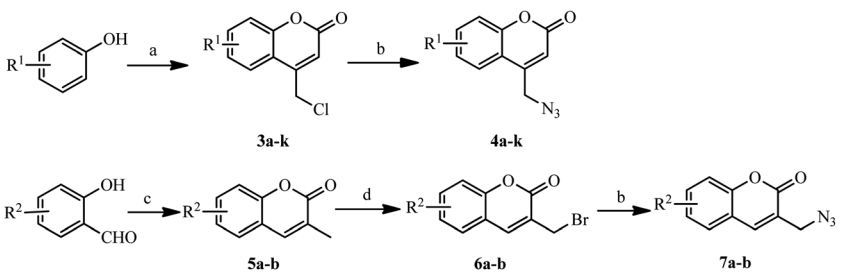
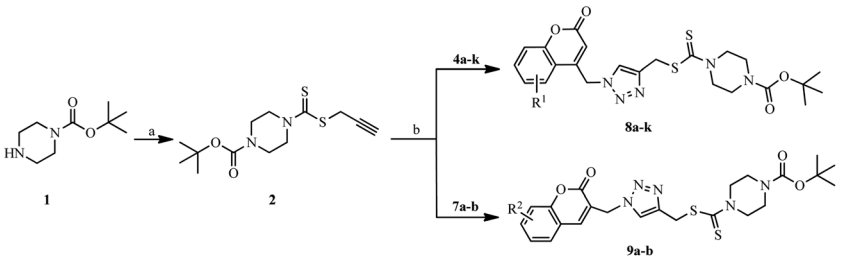


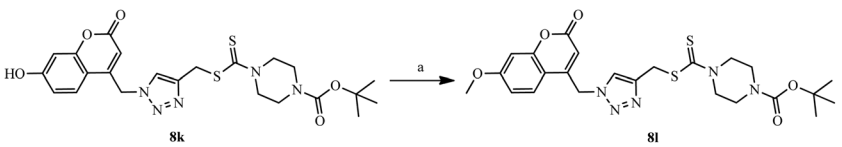
Fig. 2 Designed structures of coumarin–1,2,3-triazole–dithiocarbamate hybrids.



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_2CO_3, CH_3I, 80 °C.

intermediate **2** was efficiently prepared by following our previous described method.[19] Compounds **4a–k** were obtained by reaction of sodium azide with **3a–k** that were synthesized 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.[22] Compounds **7a–b** were synthesized from compounds **6a–b** employing 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.[23] 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.

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.[24] The demethylase activity of the recombinant LSD1 was further determined by a fluorescence-based method, using synthesized H3K4me2 as a substrate.[25] The emission wavelength for LSD1 inhibitor screening was 590 nm and the

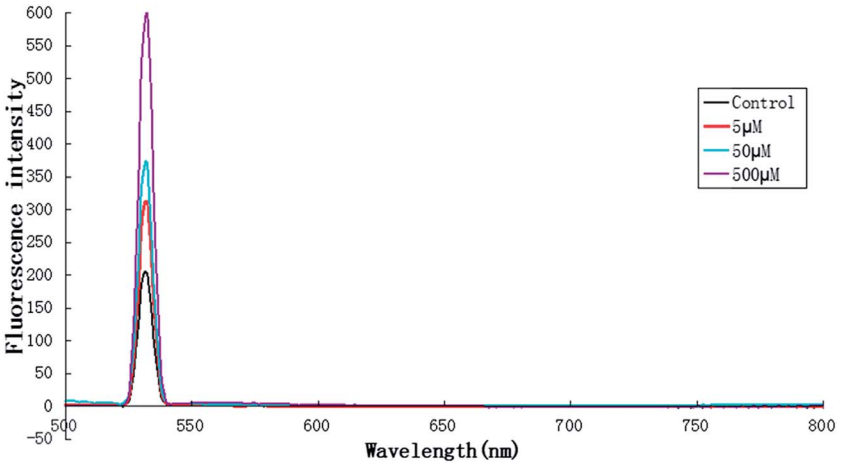


Fig. 3 Fluorescence scanning of compound **8k** at the 530 nm excitation wavelength.

excitation wavelength was 530 nm. To eliminate the possible artifacts caused by the fluorescent nature of these compounds, we did an experiment about the fluorescence scanning of compound **8k**. As shown in Fig. 3, there was no fluorescence absorption at around 590 nm (the detection wavelength), indicating that the fluorescent nature of compound **8k** had no effect towards its fluorescence absorption at around 590 nm.

In our previous work,[21] the preliminary SAR studies revealed that one carbon length between the 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 the LSD1 activity and the results are 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 μM. Among them, compound **8k** showed the most potent activity against LSD1 (IC_{50} = 0.39 μM), 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 the LSD1 inhibitory activity. Specifically, the incorporation of chloro and methyl groups into the 7-position of the coumarin nucleus (**8a** and **8f**) showed improved inhibition against LSD1 with the IC_{50} values in the nanomolar range. By contrast, compounds **8b** and **8g** with chloro atoms and methyl atoms on the 6-position of coumarin showed no inhibitory activity. Compared with **8h**, compound **8i**, with the 5,7-dihydroxy group represented excellent inhibitory activity towards LSD1 (IC_{50} = 3.00 μM). Besides, compounds **8d** and **8k** having a triazolyl group attached to the 4-position of the coumarin nucleus had great inhibitory activity towards LSD1 with the IC_{50} values of 10.33 and 0.39 μM, respectively. While for compounds **9a** and **9b**, the activity was totally lost.

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 ± 0.15 μM (74-fold more potent than that of 2-PCPA). The findings indicated the high selectivity of compound **8k** on LSD1 *in vitro*. Besides, the reversibility was also evaluated with dilution assays and dialysis experiments.[21] As shown in Fig. 4, the results indicated the reversibility of compound **8k**, compared to 2-PCPA.

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

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

Comp.	R^1	R^2	LSD1 (μM)
8a	7-Cl	—	0.67 ± 0.29
8b	6-Cl	—	>125
8c	7-F	—	84.2 ± 2.47
8d	H	—	10.33 ± 1.09
8e	7-NH_2	—	0.53 ± 0.11
8f	7-CH_3	—	0.71 ± 0.31
8g	6-CH_3	—	>125
8h	5-CH_3,7-OH	—	>125
8i	5,7-diOH	—	3.00 ± 1.32
8j	7,8-diOH	—	0.83 ± 0.23
8k	7-OH	—	0.39 ± 0.15
8l	7-OCH_3	—	0.81 ± 0.40
9a	—	H	>125
9b	—	7-OH	102.56 ± 5.23
2-PCPA	—	—	28.73 ± 1.21

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

	IC_{50} (μM)		
Compounds	LSD1	MAO-A	MAO-B
8k	0.39 ± 0.15	>1250	>1250
2-PCPA	28.73 ± 1.21	10.63 ± 1.02	5.9 ± 0.85

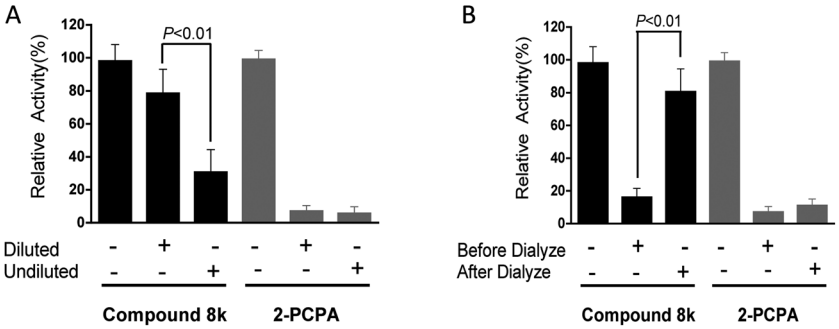


Fig. 4 The reversibility of compound **8k** to the LSD1 activity was determined by dilution assays (A) and dialysis experiments (B).

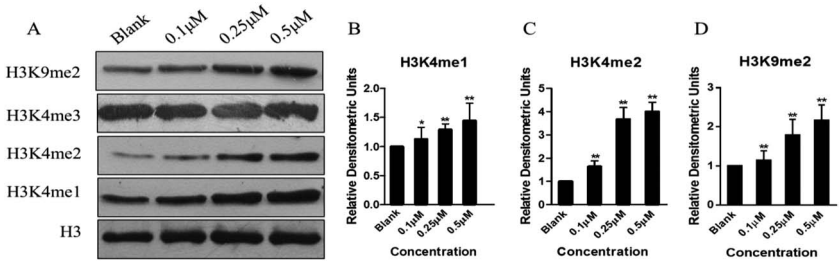


Fig. 5 Histone methylation in MGC-803 cells after treatment with compound **8k** for 48 h. (A) Expression levels of H3K4me1, H3K4me2, H3K4me3, and H3K9me2 were determined by western blot; (B) densitometry quantitation of H3K4me1 with the indicated treatment; (C) densitometry quantitation of H3K4me2 with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

compound **8k**. As shown in Fig. 5(A–D), an elevated expression of H3K4me1/2 and H3K9me2 could be found, which suggested that 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 at the recombinant level, but also active at the cell level.

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 the LSD1 inhibitory activity. Compounds **9a** and **9b** with the triazolyl group connected to the 3-position of the 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** ($IC_{50} = 0.39\ \mu M$) was 74-fold more potent than 2-PCPA and more potent than our previously published compounds.

Acknowledgements

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

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