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Pure Diastereomers of a Tranylcypromine-Based LSD1 Inhibitor: Enzyme Selectivity and In-Cell Studies

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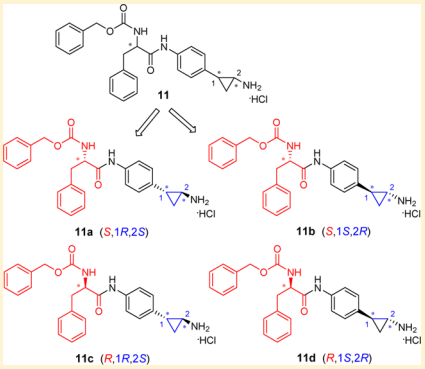
S *Supporting Information*

ABSTRACT: The pure four diastereomers (**11a−d**) of *trans*-benzyl (1-((4-(2-aminocyclopropyl)phenyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate hydrochloride **11**, previously described by us as LSD1 inhibitor, were obtained by enantiospecific synthesis/chiral HPLC separation method. Tested in LSD1 and MAO assays, **11b** (*S*,1*S*,2*R*) and **11d** (*R*,1*S*,2*R*) were the most potent isomers against LSD1 and were less active against MAO-A and practically inactive against MAO-B. In cells, all the four diastereomers induced Gfi-1b and ITGAM gene expression in NB4 cells, accordingly with their LSD1 inhibition, and **11b** and **11d** inhibited the colony forming potential in murine promyelocytic blasts.



KEYWORDS: *Epigenetics, leukemia, lysine-specific demethylase, stereoisomers, tranylcypromine*

Epigenetics influences gene expression through various mechanisms, such as methylation of cytosines in DNA, histone post-translational modifications, and nucleosome positioning along the DNA. About histone covalent modifications, such post-translational changes can be put up, removed, or interpreted by three distinct groups of enzymes, namely, writers, erasers, and readers, respectively.[1] Among the erasers, two classes of histone demethylases are known to remove methyl mark(s) from the ε-amino group of lysine residues, the lysine-specific demethylases (LSDs) and the JumonjiC (JmjC) demethylases.[1,2] LSD1 is the best studied LSD, and it is typically associated with Co-REST, a corepressor protein involved in a multicomponent repressive complex including also REST and HDAC1/2. LSD1 acts through a FAD-dependent mechanism similar to monoamine oxidases (MAOs), and specifically removes methyl groups from mono- and dimethylated Lys4 of histone H3 (H3K4me1/2). Nevertheless, in specific contexts (e.g., androgen receptor) LSD1 changes its specificity from H3K4 to H3K9,[3] thus acting either as a transcriptional corepressor or coactivator depending on its binding partners and substrates.

In addition to histones, LSD1 has also been shown to demethylate modified lysines in nonhistone proteins (including p53, STAT3, E2F1, and DNMT1),[4−6] and because of its versatility, it has been implicated in a variety of physiological as well as pathological processes, including tumorigenesis[7,8] and viral infections.[9,10] Moreover, LSD1 has been described to regulate the expression of a number of genes through its catalytic action.[4,11]

Because of the relevant role played by LSD1 mainly in tumor initiation and progression,[12−17] a number of LSD1 inhibitors has been reported to date. Some of them are related to known anti-MAO agents such as tranylcypromine (TCP, compounds **1−4** in Figure 1) or phenelzine (**5** and **6**, Figure 1), which

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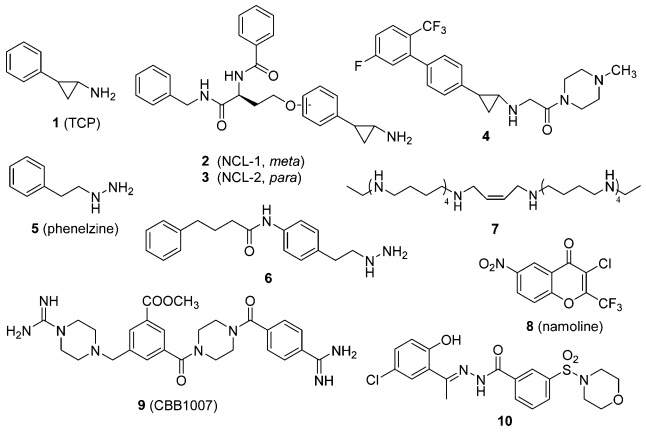


Figure 1. Structures of known irreversible (**1**–**6**) and reversible (**7**–**10**) LSD1 inhibitors.

covalently link the FAD moiety of the enzyme inhibiting its catalytic potential;[18−21] others (**7**–**10**) have been reported as reversible LSD1 inhibitors (Figure 1).[22−25] More importantly, two TCP-based LSD1 inhibitors recently entered in clinical trials for cancer treatment.[26]

Following our studies on design, synthesis, and biological evaluation of small molecule modulators of histone methylation/demethylation,[27−33] in 2010 we described some TCP-containing compounds as novel LSD1 inhibitors.[34] Among them, the benzyl (1-((4-(2-aminocyclopropyl)phenyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate hydrochloride **11** (Figure 2) was one of the most potent, with K_i = 1.3 μM, and

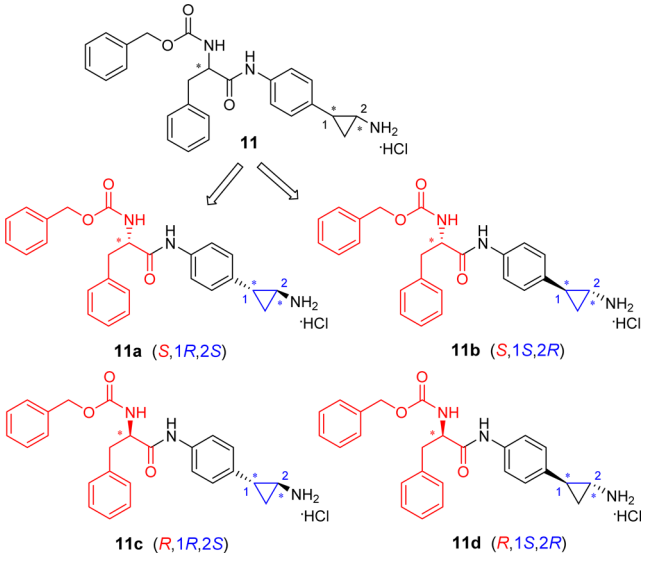


Figure 2. Chemical structures of the four stereoisomers of the prototype **11**, compounds **11a**–**d**.

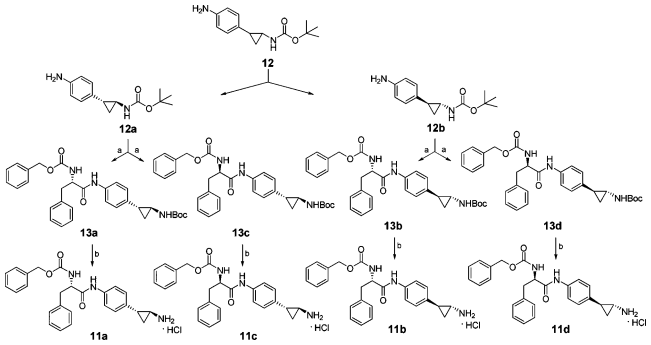
strongly enhanced the efficacy of all-trans retinoic acid (ATRA) on growth and differentiation of acute promyelocytic leukemia (APL) cells, including primary murine APL blasts.[34]

Since compound **11** contains three stereogenic centers, one at the *N*-benzyloxycarbonyl-phenylalanine moiety and two at the cyclopropyl ring, keeping these last two chiral centers fixed with a trans stereochemistry, we planned to systematically investigate the anti-LSD1 activity of the four stereoisomers of **11**, the compounds **11a**–**d** (Figure 2).

The pure diastereomers **11a**–**d** were tested against LSD1, in comparison with the isomeric mixture **11**. MAO-A and MAO-B inhibitory assays were also performed, to assess their selectivity profile. Furthermore, **11a**–**d** were evaluated in human leukemia NB4 cells to explore their capability to inhibit LSD1 in cells following transcriptional effects on genes related to LSD1 activity and/or cell differentiation. Finally, the most potent diastereomers **11b,c** were tested for their ability to affect clonogenic potential of murine promyelocytic blasts.

The preparation of the four diastereomers **11a**–**d** was based on enantiospecific synthesis starting from the trans isomers **12a** (1*S*,2*R*) and **12b** (1*R*,2*S*), which were obtained through high-performance liquid chromatography (HPLC) separation of the racemic intermediate *tert*-butyl 2-(4-aminophenyl)cyclopropyl carbamate **12**, prepared as previously described by us.[34] Both the anilino derivatives **12a** and **12b** underwent a reaction with *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI), triethylamine, *N*-hydroxybenzotriazole (HOBT), and the enantiomerically pure ((benzyloxy)-carbonyl)-L- or -D-phenylalanine in dry dichloromethane at room temperature, to obtain the *tert*-butoxycarbonyl (Boc)-protected intermediates **13a**–**d**. The cleavage of the Boc protection by **13a**–**d** through the use of 4 N hydrochloric acid in dioxane/tetrahydrofuran at room temperature afforded the four stereoisomers **11a**–**d** (Scheme 1).

Scheme 1[a]



[a]Reagents and conditions: (a) HOBT, EDCI, Et_3N, L- or D-Z-PheOH, CH_2Cl_2, 1.5 h, rt; (b) 4 N HCl, anhydrous dioxane/THF, overnight, rt.

As a result of a screening of various eluents and chiral stationary phases (CSPs), we were able to obtain a baseline HPLC separation of the two enantiomers of **12** by the cellulose-derived Chiralcel OD CSP in combination with a mobile phase consisting of a mixture of *n*-hexane/2-propanol/DEA 70:30:0.1 (v/v/v) (Figure S1b in Supporting Information). The good enantioselectivity and efficiency of the OD CSP permitted a productive scaling-up at the semipreparative level. By using a 1 cm I.D. OD column, an amount of 5 mg of sample was resolved for each HPLC run (Figure S1a in Supporting Information). In this way, 10 mg of enantiomers with an enantiomeric excess of 99% and yield >90% were isolated (Figure S1c,d in Supporting Information). In the next step, single enantiomeric forms of **12** (**12a** and **12b**, where the letters **a** and **b** denote the enantiomeric elution order) were linked to the enantiopure amino acid fragment (L- or D-*Z*-PheOH), and subsequently, the obtained stereoisomers were treated with 4 N hydrochloric acid to provide their conversion to the corresponding hydrochlorides (**11a**–**d**).

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The check of the enantiomeric and diastereomeric purity of the four stereoisomers **11a−d** was carried out by HPLC on the amylose-based Chiralpak IA CSP using the mixture *n*-hexane/ethanol/dichloromethane/DEA 50:45:5:0.1 (v/v/v/v) as a mobile phase (Figure S2b in Supporting Information).

The knowledge of the dichroic properties of the enantiomers of the *trans-tert*-butyl 2-(4-bromophenyl)cyclopropyl carbamate (Br-tPCPA),[34] prompted us to develop a multistep strategy for the absolute configuration (AC) assignment of **11a−d** based on circular dichroism (CD) and chemical correlation methods. A stepwise summary of the procedure employed is presented in Figure S2 in Supporting Information.

In a first step of the empirical approach (Figure S2a in Supporting Information), the absolute configuration of the enantiomers of **12** was assigned using the enantiomers of the parent Br-tPCPA as a reference. The CD spectra of **12** and the reference compound, recorded in ethanol, are shown in Figure S2a (Supporting Information). As expected, the enantiomeric forms of **12** exhibited specular patterns. By comparing the CD spectra of the compounds **12** to those of Br-tPCPA it appears clear that the nature of the substituent at the para position of the phenyl ring did not substantially influence the CD behavior. The findings of the CD measurements allowed us to define a parallelism between AC and CD band signs: to the first eluting enantiomer on the Chiralcel OD CSP showing all positive CD signals may be assigned the (1*S*,2*R*)-configuration and, naturally, a reversed sign of the ellipticity is expected from the second eluted (1*R*,2*S*)-enantiomer.

Once the AC of the two enantiomers of the key intermediate **12** was determined, the stereochemical assignment was extended to the stereoisomers of **11** by chemical correlation method. The reaction of connection between the amino acid derivative and the carbamate **12** and the subsequent transformation to hydrochlorides are stereoconservative processes. So, the absolute configuration of each single stereoisomer of **11** is determined by the stereochemistry of the chiral Boc derivative and the amino acid fragment (L or D) used in its synthesis. The stereochemical course of reactions was monitored by the enantio- and diastereoselective HPLC conditions described in the previous section (Figure S2b in Supporting Information).

The absolute configurations of the four stereoisomers of **11** were then assigned as follows: first eluted isomer **11a** (*S*,1*R*,2*S*), second eluted isomer **11b** (*S*,1*S*,2*R*), third eluted isomer **11c** (*R*,1*R*,2*S*), and fourth eluted isomer **11d** (*R*,1*S*,2*R*) (Figure 2).

The pure diastereomers **11a−d** were tested against LSD1 in comparison with the related mixture **11**. Data reported in Table 1 show that, among the different pure diastereoisomers **11a−d**, the two compounds carrying the 1*S*,2*R* configuration at the cyclopropyl ring displayed the highest LSD1 inhibition (**11b**, configuration, *S*,1*S*,2*R*; IC_{50} = 0.03 μM; and **11d**, configuration, *R*,1*S*,2*R*; IC_{50} = 0.05 μM), independently from the configuration shown at the *Z*-phenylalanine chiral center. The related enantiomers **11a** (configuration, *S*,1*R*,2*S*; IC_{50} = 0.36 μM) and **11c** (configuration, *R*,1*R*,2*S*; IC_{50} = 0.39 μM) were 8- and 12-fold less potent, respectively, while the mixture of isomers **11** displayed an intermediate behavior (IC_{50} = 0.15 μM). Thus, the enantiospecificity of the *Z*-phenylalanine moiety in this series of compounds is not determinant for LSD1 inhibition, whereas the configuration of the two stereogenic carbons at the cyclopropane ring was important to establish stereoselective high inhibition of LSD1.

Table 1. LSD1, MAO-A, and MAO-B Enzyme Inhibiting Activity of the Pure Diastereomers 11a−d in Comparison with Their Mixture 11

		IC_{50} (μM)[b]		
compd	AC[a]	LSD1	MAO-A	MAO-B
11	mixture	0.15	0.32	40%[c]
11a	*S*,1*R*,2*S*	0.36	0.11	42.65
11b	*S*,1*S*,2*R*	0.03	0.14	32.02
11c	*R*,1*R*,2*S*	0.39	0.20	14%[c]
11d	*R*,1*S*,2*R*	0.05	0.22	33%[c]

[a]AC, absolute configuration. [b]Data represent mean values of at least two separate experiments in duplicate; the error is within ±10%. [c]Percentage of inhibition at 100 μM.

Against the human MAO enzymes (Table 1), the stereoisomers **11a−d** displayed submicromolar inhibition against MAO-A, with a selectivity index for LSD1 ranging from 0.5 to 4.6. Interestingly, **11a** and **11b** (with the L-*Z*-Phe moiety, *S* configuration) displayed low inhibition versus MAO-B, being 117- and 970-fold selective for LSD1, respectively, whereas **11c** and **11d** (with the D-*Z*-Phe moiety, *R* configuration) were almost totally inactive. Thus, in these compounds the presence of the *R* configuration at the *Z*-amino acid moiety decreases the inhibiting activity against MAO-B and increases the LSD1 selectivity.

LSD1 can control gene expression through demethylation. Growth factor independence 1 (Gfi-1) is a transcription factor that regulates the adult hematopoietic stem and progenitor cells development. In particular, it is able to repress the expression of genes implicated in cell survival, proliferation, and differentiation. In erythroid cells, Gfi-1b is part of a large protein complex containing LSD1 and CoREST,[35] and LSD1 depletion derepresses Gfi-1b targets in lineage-specific patterns.[35] Alterations of Gfi-1 expression and function have been associated with neutropenia, allergy, autoimmunity, and hyperinflammatory responses, as well as to lymphoma and leukemia development.[36] Integrin alpha M (ITGAM), also known as CR3A and cluster of differentiation molecule 11B (CD11B), is a typical marker of differentiation in leukemia cells. Compound **11** was shown as able to enhance the expression of the differentiation marker CD11B by ATRA in NB4 cells and to induce differentiation of murine promyelocytic leukemia (APL) blasts up to 77.9%,[34] thus demonstrating its cell differentiation properties in leukemia.

These findings prompted us to assess the capability of **11a−d** to induce Gfi-1b and ITGAM gene expression in human APL NB4 cells, in comparison with the reference mixture **11**. For this scope, NB4 cells were incubated with the inhibitors at a concentration corresponding to their biochemical IC_{50} value. After 24 h, the mRNA expression of the Gfi-1b and ITGAM genes was measured by quantitative RT-PCR and expressed as fold-induction respect to DMSO used as a control. Data depicted in Figure 3 show that **11a−d** were able to induce gene expression, increasing Gfi-1b expression up to 5/6-fold (**11c** and **11d**) or up-regulating the ITGAM (CD11B) gene up to 3.7-fold (**11d**). As expected, no significant differences were observed between **11a−d** since each compound was tested at its respective biochemical IC_{50} on LSD1.

Finally, the most potent compounds **11b** and **11d** were tested for their ability to inhibit colony formation of murine promyelocytic blasts at the fixed concentration of 0.25 μM in comparison with the isomeric mixture **11**. After 7 days of

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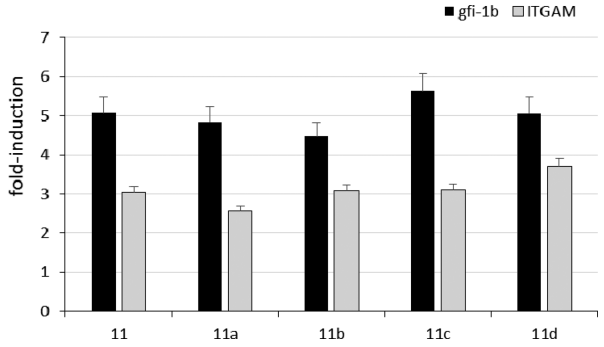

Figure 3. Gfi-1b and ITGAM gene expression modulation by **11a–d** in NB4 cells. Compounds were tested at their anti-LSD1 IC_{50} values. Data are expressed as average fold induction versus the vehicle (DMSO).

semisolid culture, **11b** and **11d** inhibited about 59% colony formation (Table 2), while **11** exhibited a lower value (about 37%), in line with the lower biochemical potency.

Table 2. Anticlonogenic Activity of Selected 11 Compounds

compd	AC[a]	colony forming unit assay, % of inhibition at 0.25 μM[b]
11	mixture	36.8
11b	*S*,1*S*,2*R*	59.2
11d	*R*,1*S*,2*R*	59.6

[a]AC, absolute configuration. [b]Data represent mean values of at least two separate experiments in duplicate; the error is within ±10%.

In summary, we previously described the *Z*-PheCONH-TCP hydrochloride **11** as an efficient inhibitor of LSD1, also showing differentiating effects in APL cells including primary murine APL blasts.[34]

Since **11** has three chiral centers and is a mixture of different diastereomers, we performed chiral separation and enantiospecific synthesis to obtain the single pure diastereomers **11a–d** and tested them against LSD1 and MAO enzymes. In the anti-LSD1 assay, the two diastereomers bearing the 1*S*,2*R* configuration at the cyclopropyl ring, i.e., **11b** (*S*,1*S*,2*R*) and **11d** (*R*,1*S*,2*R*), were 8.2- and 11-fold more potent than the related **11a** (*S*,1*R*,2*S*) and **11c** (*R*,1*R*,2*S*), respectively, highlighting that in this series of compounds the stereochemistry of the cyclopropane ring, and not that of the *Z*-amino acid residue, is crucial for the inhibiting activity. Compounds **11b** and **11d** displayed also lower inhibiting potency against MAO-A and scarce or no activity against MAO-B, thus resulting in LSD1-selective. The four diastereomers **11a–d**, when tested in human APL NB4 cells at their biochemical IC_{50}s to determine the capability to induce Gfi-1b and ITGAM gene expression as a marker of LSD1 inhibition in cells, highly increased the expression of both the genes. Finally, **11b** and **11d**, the two most potent diastereomers against LSD1 in vitro, inhibited the colony forming potential of murine promyelocytic blasts by 59%. These compounds will be further investigated as antiproliferative and cytodifferentiating agents in a panel of cancer cells.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental section: synthetic procedures to obtain **11a–d**. Chemical and physical data of **11a–d**. Analytical details and CD spectra for enantiomeric separation of **12**; assignment of the absolute configurations of **11a–d**. LSD1 and MAOs inhibitory assays. Gene modulation assay. Clonogenic assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

APL, acute promyelocytic leukemia; ATRA, all-trans-retinoic acid; Br-tPCPA, *trans*-2-(4-bromophenyl)cyclopropan-1-amine; CD, circular dichroism; CD11B, cluster of differentiation molecule 11B; CoREST, corepressor for element-1-silencing transcription factor; CSP, chiral stationary phase; DEA, diethylamine; EDCI, *N*-(3-(dimethylamino)propyl)-*N*′-ethyl-carbo diimide hydrochloride; E2F1, transcription factor E2F1; Gfi-1b, growth factor independent 1B transcription repressor; HDAC, histone deacetylase; HOBT, *N*-hydroxybenzotriazole; HPLC, high performance liquid chromatography; ITGAM, integrin alpha M; JmjC, JumonjiC demethylase; LSD, lysine specific demethylase; MAO, monoamine oxidase; OD, optical density; STAT3, signal transducer and activator of transcription 3; TCP, tranylcypromine

■ REFERENCES

(1) Helin, K.; Dhanak, D. Chromatin proteins and modifications as drug targets. *Nature* **2013**, *502*, 480–488.
(2) Rotili, D.; Mai, A. Targeting histone demethylases: A new avenue for the fight against cancer. *Genes Cancer* **2011**, *2*, 663–679.
(3) Metzger, E.; Wissmann, M.; Yin, N.; Muller, J. M.; Schneider, R.; Peters, A. H.; Gunther, T.; Buettner, R.; Schule, R. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* **2005**, *437*, 436–439.
(4) Huang, J.; Sengupta, R.; Espejo, A. B.; Lee, M. G.; Dorsey, J. A.; Richter, M.; Opravil, S.; Shiekhattar, R.; Bedford, M. T.; Jenuwein, T.; Berger, S. L. p53 is regulated by the lysine demethylase LSD1. *Nature* **2007**, *449*, 105–108.
(5) Kontaki, H.; Talianidis, I. Lysine methylation regulates E2F1-induced cell death. *Mol. Cell* **2010**, *39*, 152–160.
(6) Wang, J.; Hevi, S.; Kurash, J. K.; Lei, H.; Gay, F.; Bajko, J.; Su, H.; Sun, W.; Chang, H.; Xu, G.; Gaudet, F.; Li, E.; Chen, T. The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nat. Genet.* **2009**, *41*, 125–129.
(7) Højfeldt, J. W.; Agger, K.; Helin, K. Histone lysine demethylases as target for anticancer therapy. *Nat. Rev. Drug Discovery* **2013**, *12*, 917–930.
(8) Lynch, J. T.; Harris, W. J.; Somervaille, T. C. *Expert Opin. Ther. Targets* **2012**, *16*, 1239–1249.

(9) Liang, Y.; Vogel, J. L.; Narayanan, A.; Peng, H.; Kristie, T. M. Inhibition of the histone demethylase LSD1 blocks α-herpes virus lytic replication and reactivation from latency. *Nat. Med.* **2009**, *15*, 1312−1317.
(10) Andrisani, O. M. Deregulation of epigenetic mechanisms by the hepatitis B virus X protein in hepatocarcinogenesis. *Viruses* **2013**, *5*, 858−872.
(11) Lee, M. G.; Wynder, C.; Cooch, N.; Shiekhattar, R. An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. *Nature* **2005**, *437*, 432−435.
(12) Schulte, J. H.; Lim, S.; Schramm, A.; Friedrichs, N.; Koster, J.; Versteeg, R.; Ora, I.; Pajtler, K.; Klein-Hitpass, L.; Kuhfittig-Kulle, S.; Metzger, E.; Schule, R.; Eggert, A.; Buettner, R.; Kirfel, J. Lysine-specificdemethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy. *Cancer Res.* **2009**, *69*, 2065−2071.
(13) Wissmann, M.; Yin, N.; Muller, J. M.; Greschik, H.; Fodor, B. D.; Jenuwein, T.; Vogler, C.; Schneider, R.; Gunther, T.; Buettner, R.; Metzger, E.; Schule, R. Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. *Nat. Cell Biol.* **2007**, *9*, 347−353.
(14) Scoumanne, A.; Chen, X. The lysine-specific demethylase 1 is required for cell proliferation in both p53-dependent and -independent manners. *J. Biol. Chem.* **2007**, *282*, 15471−15475.
(15) Lim, S.; Janzer, A.; Becker, A.; Zimmer, A.; Sch€ule, R.; Buettner, R.; Kirfel, J. Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology. *Carcinogenesis* **2010**, *31*, 512−520.
(16) Wang, Y.; Zhang, H.; Chen, Y.; Sun, Y.; Yang, F.; Yu, W.; Liang, J.; Sun, L.; Yang, X.; Shi, L.; Li, R.; Li, Y.; Zhang, Y.; Li, Q.; Yi, X.; Shang, Y. LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. *Cell* **2009**, *138*, 660−672.
(17) Harris, W. J.; Huang, X.; Lynch, J. T.; Spencer, G. J.; Hitchin, J. R.; Li, Y.; Ciceri, F.; Blaser, J. G.; Greystoke, B. F.; Jordan, A. M.; Miller, C. J.; Ogilvie, D. J.; Somervaille, T. C. The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells. *Cancer Cell* **2012**, *21*, 473−487.
(18) Schmidt, D. M.; McCafferty, D. G. *trans*-2-Phenylcyclopropylamine is a mechanism-based inactivator of the histone demethylase LSD1. *Biochemistry* **2007**, *46*, 4408−4416.
(19) Ueda, R.; Suzuki, T.; Mino, K.; Tsumoto, H.; Nakagawa, H.; Hasegawa, M.; Sasaki, R.; Mizukami, T.; Miyata, N. Identification of cell-active lysine specific demethylase 1-selective inhibitors. *J. Am. Chem. Soc.* **2009**, *131*, 17536−17537.
(20) Baker, J. A.; Castro Palomino, J.; Fyfe, M. C. T.; Maes, T.; Martinell Pedemonte, M. Lysine demethylase inhibitors for treatment of myeloproliferative disorders. PCT Int. Appl. WO 2012107498 A1 20120816, 2012.
(21) Prusevich, P.; Kalin, J. H.; Ming, S. A.; Basso, M.; Givens, J.; Li, X.; Hu, J.; Taylor, M. S.; Cieniewicz, A. M.; Hsiao, P. Y.; Huang, R.; Roberson, H.; Adejola, N.; Avery, L. B.; Casero, R. A., Jr.; Taverna, S. D.; Qian, J.; Tackett, A. J.; Ratan, R. R.; McDonald, O. G.; Feinberg, A. P.; Cole, P. A. A selective phenelzine analogue inhibitor of histone demethylase LSD1. *ACS Chem. Biol.* **2014**, *9*, 1284−1293.
(22) Huang, Y.; Stewart, T. M.; Wu, Y.; Baylin, S. B.; Marton, L. J.; Perkins, B.; Jones, R. J.; Woster, P. M.; Casero, R. A., Jr. Novelolig oamine analogues inhibit lysine-specific demethylase 1 and induce reexpression of epigenetically silenced genes. *Clin. Cancer Res.* **2009**, *15*, 7217−7228.
(23) Willmann, D.; Lim, S.; Wetzel, S.; Metzger, E.; Jandausch, A.; Wilk, W.; Jung, M.; Forne, I.; Imhof, A.; Janzer, A.; Kirfel, J.; Waldmann, H.; Schüle, R.; Buettner, R. Impairment of prostate cancer cell growth by a selective and reversible lysine-specific demethylase 1 inhibitor. *Int. J. Cancer* **2012**, *131*, 2704−2709.
(24) Wang, J.; Lu, F.; Ren, Q.; Sun, H.; Xu, Z.; Lan, R.; Liu, Y.; Ward, D.; Quan, J.; Ye, T.; Zhang, H. Novel histone demethylase LSD1 inhibitors selectively target cancer cells with pluripotent stem cell properties. *Cancer Res.* **2011**, *71*, 7238−7249.
(25) Sorna, V.; Theisen, E. R.; Stephens, B.; Warner, S. L.; Bearss, D. J.; Vankayalapati, H.; Sharma, S. High-throughput virtual screening identifies novel *N*′-(1-phenylethylidene)-benzohydrazides as potent, specific, and reversible LSD1 inhibitors. *J. Med. Chem.* **2013**, *56*, 9496−9508.
(26) http://clinicaltrials.gov/ct2/results?term=lysine+demethylase+inhibitor&Search=Search.
(27) Mai, A.; Valente, S.; Cheng, D.; Perrone, A.; Ragno, R.; Simeoni, S.; Sbardella, G.; Brosch, G.; Nebbioso, A.; Conte, M.; Altucci, L.; Bedford, M. T. Synthesis and biological validation of novel synthetic histone/protein methyltranferase inhibitors. *ChemMedChem* **2007**, *2*, 987−991.
(28) Mai, A.; Cheng, D.; Bedford, M. T.; Valente, S.; Nebbioso, A.; Perrone, A.; Brosch, G.; Sbardella, G.; De Bellis, F.; Miceli, M.; Altucci, L. Epigenetic multiple ligands: mixed histone/protein methyltransferase, acetyltransferase, and class III deacetylase (sirtuin) inhibitors. *J. Med. Chem.* **2008**, *51*, 2279−2290.
(29) Cheng, D.; Valente, S.; Castellano, S.; Sbardella, G.; Di Santo, R.; Costi, R.; Bedford, M. T.; Mai, A. Novel 3,5-bis-(bromohydroxybenzylidene)piperidin-4-ones as coactivator-associated arginine methyltransferase 1 inhibitors: enzyme selectivity and cellular activity. *J. Med. Chem.* **2011**, *54*, 4928−4932.
(30) Upadhyay, A. K.; Rotili, D.; Han, J. W.; Hu, R.; Chang, Y.; Labella, D.; Zhang, X.; Yoon, Y. S.; Mai, A.; Cheng, X. An analog of BIX-01294 selectively inhibits a family of histone H3 lysine 9 Jumonji demethylases. *J. Mol. Biol.* **2012**, *416*, 319−327.
(31) Valente, S.; Lepore, I.; Dell'Aversana, C.; Tardugno, M.; Castellano, S.; Sbardella, G.; Tomassi, S.; Di Maro, S.; Novellino, E.; Di Santo, R.; Costi, R.; Altucci, L.; Mai, A. Identification of PR-SET7 and EZH2 selective inhibitors inducing cell death in human leukemia U937 cells. *Biochimie* **2012**, *94*, 2308−2313.
(32) Rotili, D.; Tomassi, S.; Conte, M.; Benedetti, R.; Tortorici, M.; Ciossani, G.; Valente, S.; Marrocco, B.; Labella, D.; Novellino, E.; Mattevi, A.; Altucci, L.; Tumber, A.; Yapp, C.; King, O. N.; Hopkinson, R. J.; Kawamura, A.; Schofield, C. J.; Mai, A. Pan-histone demethylase inhibitors simultaneously targeting Jumonji C and lysine-specific demethylases display high anticancer activities. *J. Med. Chem.* **2014**, *57*, 42−55.
(33) Vianello, P.; Botrugno, O. A.; Cappa, A.; Ciossani, G.; Dessanti, P.; Mai, A.; Mattevi, A.; Meroni, G.; Minucci, S.; Thaler, F.; Tortorici, M.; Trifiró, P.; Valente, S.; Villa, M.; Varasi, M.; Mercurio, C. *Eur. J. Med. Chem.* **2014**, *86*, 352−363.
(34) Binda, C.; Valente, S.; Romanenghi, M.; Pilotto, S.; Cirilli, R.; Karytinos, A.; Ciossani, G.; Botrugno, O. A.; Forneris, F.; Tardugno, M.; Edmondson, D. E.; Minucci, S.; Mattevi, A.; Mai, A. Biochemical, structural, and biological evaluation of tranylcypromine derivatives as inhibitors of histone demethylases LSD1 and LSD2. *J. Am. Chem. Soc.* **2010**, *132*, 6827−6833.
(35) Saleque, S.; Kim, J.; Rooke, H. M.; Orkin, S. H. Epigenetic regulation of hematopoietic differentiation by Gfi-1 and Gfi-1b is mediated by the cofactors CoREST and LSD1. *Mol. Cell* **2007**, *27*, 562−572.
(36) van der Meer, L. T.; Jansen, J. H.; van der Reijden, B. A. Gfi1 and Gfi1b: key regulators of hematopoiesis. *Leukemia* **2010**, *24*, 1834−1843.