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Identification of catechols as histone–lysine demethylase inhibitors

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ABSTRACT

Identification of inhibitors of histone–lysine demethylase (HDM) enzymes is important because of their involvement in the development of cancer. An ELISA-based assay was developed for identification of inhibitors of the HDM KDM4C in a natural products library. Based on one of the hits with affinity in the low μM range (1, a catechol), a subset of structurally related compounds was selected and tested against a panel of HDMs. In this subset, two inhibitors (2 and 10) had comparable affinities towards KDM4C and KDM6A but no effect on PHF8. One inhibitor restored H3K9me3 levels in KDM4C transfected U2-OS cells.

1. Introduction

Histone lysine methylation has been identified as an important epigenetic marker. Methylation of specific lysine residues in the histone tails can act as either activators or repressors of DNA transcription and thus has a key role in the regulation of specific genes [1]. Histone–lysine demethylase (HDM) enzymes have an important role in maintaining the correct transcription levels and consequently, they are central in epigenetic research. One class of HDMs are the Jumonji-C-Domain (JmjC) containing HDMs that are 2-oxoglutarate (2OG) and Fe dependent enzymes belonging to the 2OG dependent oxygenase super family [2].

The HDM KDM4C (GASC1/JMJD2C) has been identified as a putative oncogene as it is upregulated in different types of cancer [3], and inhibition of this enzyme has been shown to arrest tumor cell proliferation [4]. The HDM KDM6A (UTX) has been identified as a regulator of HOX genes that are imperative during cellular differentiation [5]. Further work has shown that KDM6A most likely acts as a tumor repressor [6]. Thus, an inhibitor should be selective for KDM4C over KDM6A.

Mutations in the PHF8 HDM impairing its catalytic activity have been shown in X-linked mental retardation patients [7] and recently, overexpression of PHF8 was shown in prostate cancer cells [8]. Thus, depending on the context of expression pattern, PHF8 is another member of the JmjC HDM family towards which, inhibitors targeting the putative oncogenes should be selective. The three enzymes are active towards different methylation states, with KDM4C primarily demethylating trimethylated lysine 9 on histone 3 (H3K9me3), KDM6A demethylating H3K27me3 and PHF8 demethylating H3K9me2.

A number of HDM inhibitors have been reported [9], including N-oxalyl-glycine (NOG) based derivatives with increased affinity and selectivity [10], inhibitors that are based on 2,4-pyridine di-carboxylic acid (2,4-PDCA) [11], hydroxamic acids [12,13], and recently, a KDM4C selective substrate-based inhibitor [14]. In addition to the reported synthesis of de-novo compounds, screening of a number of different compound libraries against KDM4E have been reported [15–17]. Whereas most screening studies have been focused on identification of small molecule inhibitors of a single HDM, a recent paper demonstrates that selectivity can indeed be obtained, as 2,4-PDCA was shown to have significant selectivity for KDM4C over KDM6A [18].

In this study, a 640 member diversity-optimized natural product library was screened for inhibitors of KDM4C. Based on one of the hits, a subset of compounds were selected and screened

Abbreviations: cc, catalytic core; ccKDM4C, catalytic core of KDM4C; ccKDM6A, catalytic core of KDM6A; 2,4-PDCA, 2,4-pyridine di-carboxylic acid; 2OG, 2-oxoglutarate; ELISA, enzyme-linked immunosorbent assay; FDH, formaldehyde dehydrogenase; H3K9, histone 3 lysine 9; HDM, histone lysine demethylase; HRP, horse radish peroxidase; JmjC, Jumonji C; NOG, N-oxalylglycine

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against KDM4C, KDM6A, and PHF8 to elucidate possible differences in inhibitor selectivity between closely related HDM enzymes, followed by *in-cell* evaluation of a selected hit.

2. Materials and methods

2.1. Reagents and chemicals

All chemicals used for buffers, co-factors, lysozyme, DNAse, formaldehyde dehydrogenase from Pseudomonas putida, N-oxalylglycine, 2,4-pyridine di-carboxylic acid, TMB Elisa detection solution, BSA, Tween-20, a-cyano-4-hydroxy cinnamic acid and Hoechst staining (bisBenzimide H 33342), were obtained from Sigma-Aldrich, Denmark. Solvents for MALDI-TOF were of HPLC-grade and from Merck, Denmark. EDTA free protease inhibitor cocktail tablets used in protein purification and FuGENE HD for transfection were from Roche. Histone tail fragments Biotin-H3(1-24)K9me3, H3(7-14)K9me3, H3(1-24)K4me3K9me2 and H3K27me3 synthetic peptide substrates were purchased from Peptide 2.0, USA or generously donated by Novo Nordisk A/S, Denmark. Reacti-bind streptavidin functionalized 96-well plates were from Pierce, rabbit anti-mouse-HRP conjugate was purchased from DAKO and anti-H3K9me2 mouse antibody as well as primary antibodies for cell based assays; rabbit polyclonal anti-H3K9me3 (ab8898) and rabbit polyclonal anti-H3K9me1 (ab9045), were from Abcam. The 640 compound Natural Product Library was purchased from TimTec. The plasmid pET His KDM4C1-349 for recombinant expression of the His-tagged catalytic core of human KDM4C (cc-KDM4C, 1-349) and pCMV-HA-KDM4C for ectopic expression of HA-tagged full-length human KDM4C [4] were generously donated by the Biotech Research and Innovation Centre (BRIC), Denmark. The pOPIN vector J was a gift from the Oxford Protein Production Facility, Great Britain. Compounds **1**–**9**, **11**–**13** were purchased from TimTec, while compounds **10**, **14**–**18** were donated by Novo-Nordisk A/S. Dulbecco's Modified Eagle Medium (DMEM) and secondary antibodies for cells based assays, Alexa Flour®488 anti-mouse and Alexa Flour®594 anti-rabbit was purchased from Invitrogen. Purified mouse monoclonal anti-HA.11 (Clone 16B12) was purchased from Covance – Biosite, Sweden.

2.2. Screening of natural product library applying the ELISA assay

Streptavidin plates were washed with buffer A (50 mM Hepes, 50 mM NaCl pH 7.4) and biotinylated peptide (10 μg/ml) was immobilized over night at 4 °C. The following morning the plate was washed and blocked for 2 h at r.t. with buffer B (50 mM HEPES, 50 mM NaCl, 0.05% Tween-20 and 0.1% BSA pH 7.4). In a separate 96-well plate, the catalytic core of KDM4C (ccKDM4C) was diluted to 250 nM in reaction buffer (final volume 100 μl) and 5 μl (1 mg/ml) of the putative inhibitors were added to the appropriate wells. Four wells were used as a negative control without enzyme and four wells were used as a positive control with 5% DMSO. The controls were used for calculating Z'-values for each of the eight screened plates (see Supplementary data). The plate was incubated on ice for 1 h. The blocked functionalized plate was washed with buffer A and 100 μl enzyme/inhibitor mixture was added to each well and the plate was incubated for 1.5 h at r.t. followed by wash with buffer A. The primary anti-H3K9me2 antibody was diluted 1:1000 into buffer B, added to the plate and incubated for 1 h at r.t. The plate was washed with buffer A and the secondary antibody was added as described for the primary antibody and incubated for 1 h at r.t. Following the second incubation, the plate was thoroughly washed with buffer A and 100 μl of tetra-methylbenzidine solution (Sigma) was added to each well until a desired color intensity was obtained. The reaction was quenched by addition of 50 μl 1 M HCl to each well and the absorbance was recorded at 450 nM on a Safire microplate-reader (Tecan).

2.3. FDH assay

The FDH assays were carried out essentially as described elsewhere [19], for details see Supplementary data.

2.4. Cell based assay

Human U2-OS osteosarcoma cells were cultured in monolayer in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (DMEM/FBS/PS). 1×10^3 U2-OS cells were seeded in 100 μL in each well of a 96 well NUNC flat bottomed transparent plate 12 h before transfection. Transfection was performed using FuGENE HD according to the manufacturer's instructions.

Compound **10** was dissolved to 250 mM in 1 M sterile Tris–HCl pH 9.0 and a twofold dilution series was prepared freshly for each individual experiment. 5 μL from the dilution series was mixed with 95 μL DMEM/FBS/PS and the media was exchanged. Cells were incubated for 23 h and fixed with 4% paraformaldehyde in Phosphate Buffered Saline (PBS), permeabilished with 0.1% Triton X100 in PBS and blocked for 1 h in DMEM/FBS/PS. Cells were stained with mouse anti-HA.11 antibody for detection of transfected cells, and either rabbit anti-H3K9me3 antibody or rabbit anti-H3K9me1 antibody for detection of H3K9 methylation level over night at 4 °C. Cells were washed with PBS and stained with Hoecst for nuclei detection and secondary antibodies, Alexa Flour®488 anti-mouse and Alexa Flour®594 anti-rabbit at 4 °C in the dark. Cells were washed with PBS and left at 4 °C in PBS before imaging. The cells were imaged on an IN Cell Analyzer 1000 (GE Healthcare), and 6 images per well were acquired using a 10× objective to count ~2000 cells per well.

Acquired images were analyzed using the IN Cell Analyzer Workstation 3.6 software (GE Healthcare). Average intensity/nuclei for each methyl mark were normalized against non-transfected cells and transfected cells above (for H3K9me1 staining) and below (for H3K9me3 staining) this threshold were counted and plotted as percentage of the total number of transfected cells. For each concentration of inhibitor, the intensity was measured on an average of 350–750 transfected cells. Each experiment was performed in triplicate on three different days.

Preparation of cover slips was performed essentially as described above. The stained cover slips were washed one time in de-mineralized water and dried over night in the dark. The cover slips were mounted on glass slides and sealed with transparent nail polish. The cover slips were analyzed with a Carl Zeiss confocal microscope and LSM 5.1 software 3.

3. Results and discussion

3.1. Screening of natural product library for inhibitors of ccKDM4C.

Using a 24-meric N-terminally biotinylated H3K9me3 peptide, an ELISA-based assay was developed, where the product formation was detected using a monoclonal antibody directed against the di-methylated product. Product formation was detected using a Horse Radish Peroxidase (HRP) conjugated secondary antibody. A test of the assay revealed that Z'-values [20] >0.5 could be obtained with the described setup and the assay was validated against the known KDM4C inhibitor 2,4-PDCA (Fig. S4). The assay was conducted at 250 nM ccKDM4C, which is 10-fold lower compared to the in-house FDH assay setup.

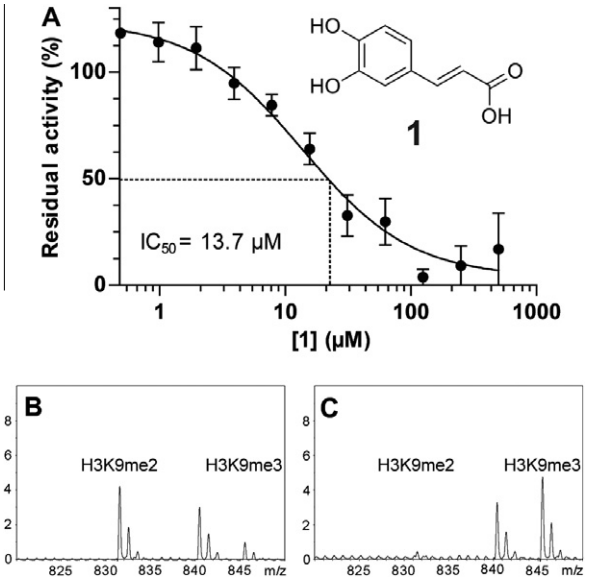


Fig. 1. The inhibition of KDM4C by **1** determined using FDH assay and verified by MALDI-TOF-MS. (A) Inhibition curve of **1** against KDM4C at 25 μM H3(7-14)K9me3, showing an IC_{50} of 13.7 μM. Inset shows the structure of compound **1**. (B) MALDI-TOF mass spectrum of a reaction-mixture consisting of KDM4C and 25 μM H3(7-14)K9me3 (m/z = 846) without inhibitor. The spectrum shows formation of H3(7-14)K9me2 (m/z = 832). (C) MALDI-TOF mass spectrum with a mixture identical to that in panel B, but with 50 μM **1** added, showing that the inhibitor arrests the product formation. The peak at m/z = 840 results from sample contamination.

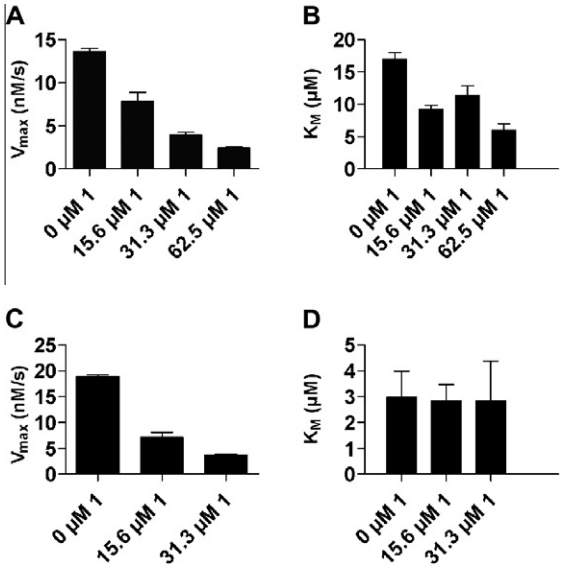


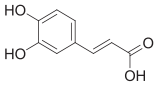
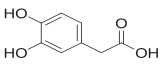
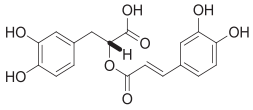
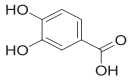
Fig. 2. Analysis of the mechanism of action of **10**. Top panel (A and B) V_{max} and K_M values for fixed 2OG concentrations and varying H3(7-14)K9me3 concentrations at 0 μM **10**, 15.6 μM **10**, 31.25 μM **10** and 62.5 μM **10**. Bottom panel (C and D) V_{max} and K_m values for fixed H3(7-14)K9me3 concentrations and varying 2OG concentrations at 0 μM **10**, 15.6 μM **10** and 31.25 μM **10**. Full Michaelis–Menten curves are found in Supplementary data.

The technique was used for screening of potential inhibitors of ccKDM4C in a commercially available diversity optimized natural-products library containing 640 compounds. A number of hits in the library were identified (see Fig. S5 and Table S1), and based on structural features, a subset of 21 compounds were subsequently tested in the standard FDH coupled assay (Table S1). This approach identified compound 1 as an inhibitor of ccKDM4C with an IC_{50} of 13.7 μM (Fig. 1).

3.2. Structurally similar compounds to compound 1 tested against other HDMs.

A second set consisting of 18 compounds, selected based on their structural similarity to **1** (cf Table 1 and Table S2), was tested against three different JmjC HDM catalytic cores, namely ccKDM4C, ccKDM6A, and a truncated version of PHF8 (tPHF8), comprising the PHD domain and the catalytic core, (see Supplementary data). The enzymes were selected as representa-

Table 1
Inhibition by catechol containing compounds from analysis of the 18 compounds selected based on their similarity to compound **1**.

		KDM4C		PHF-8		KMD6A	
		Single point inhibition*	IC_{50}/Fe^{2+} ratio[a]	Single point inhibition*	IC_{50}/Fe^{2+} ratio[a]	Single point inhibition*	IC_{50}/Fe^{2+} ratio[a]
1		+	13.7 ± 1.3/2.8	–	NI	+	5.5 ± 1.1
2		+	90 ± 1.4/1.5	–	NI	+	7.2 ± 1.2
6		+	64.6 ± 1.4/>6	–	NI	+	20 ± 1.4
10		+	52 ± 1.4/2.5	–	NI	+	12 ± 1.2

NI: No inhibition.
[a] The ratio between IC_{50} values determined at 100 μM and 20 μM Fe^{2+}, respectively.
* Classification of the compound as an inhibitor based on screening at a single high inhibitor concentration as shown in Fig. S6.

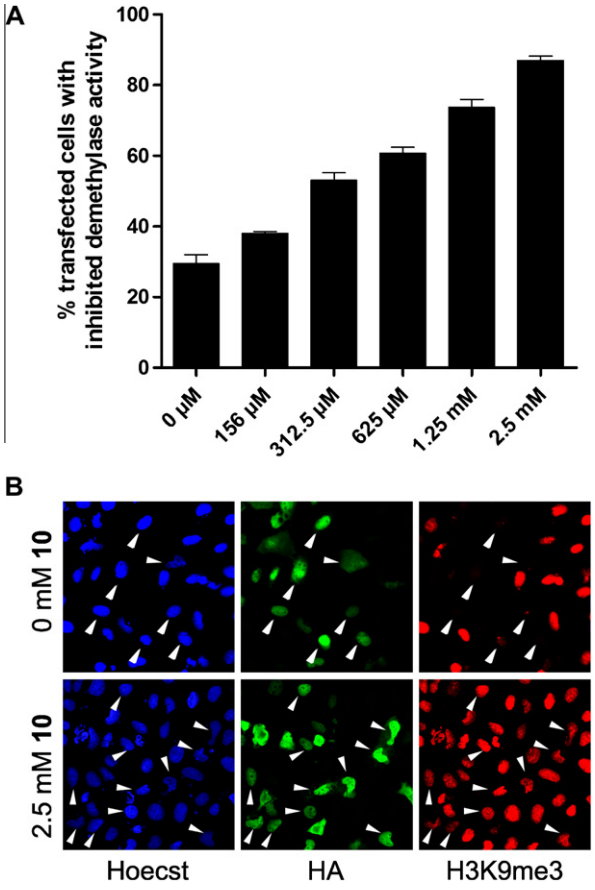


Fig. 3. Cell-based activity of compound **10** in U2-OS cells. (A) Percentage of KDM4C transfected U2-OS cells at different concentrations of **10** with H3K9me3 levels at the threshold value. The threshold value for the H3K9me3 signal is calculated as the average intensity/nuclei of non transfected cells, and represent the base level of H3K9me3 in the U2-OS cells. At increasing concentrations of **10**, the percentage of KDM4C transfected cells with H3K9me3 signals at the base level of H3K9me3 increases, indicating in cell inhibition of KDM4C. (B) U2-OS cells transfected with HA-tagged KDM4C in the abscence or presence of 2.5 mM **10**. White arrows indicate cells with changed H3K9me3 levels.

tives from different HDM subfamilies with the aim of identifying selective inhibitors for different HDM subfamilies. Initially, the 18 selected compounds were screened against the enzyme ensemble at a single concentration of 250 μM in the FDH assay (see Fig. S6). IC_{50} curves were subsequently determined for compounds that exhibited inhibition in the single-point determinations.

In this second set of compounds, compounds **2**, **6** and **10** were identified as inhibitors of ccKDM4C and ccKDM6A (Table 1), while interestingly; none of the compounds affected the activity of tPHF8. However, the IC_{50} value of compound **6** in the inhibition of ccKDM4C turned out to be dependent on the Fe^{2+} concentration. Thus, it is likely the inhibitor chelates Fe^{2+} and thereby inhibits the protein in an unspecific manner (Table 1). The compounds had higher affinity for ccKDM6A, indicating that these homologous enzymes are sufficiently different to achieve selectivity using the compounds identified in this screen as leads. It is of particular interest that compound **2** is approximately 10-fold less potent towards ccKDM4C compared to **1**, while both compounds were equally potent towards ccKDM6A. This is also true for **10**, which was less potent towards both enzymes, but at the same time, the smaller molecule is more suited for engineering in order to achieve selectivity.

3.3. Mode of action of compound *10*

Catechols are known inhibitors of 2OG dependent enzymes, and as highlighted in a recent review, the inhibition mechanism of this compound class has been shown to be complex [21]. It is, however, interesting that no inhibition of PHF8 was observed, indicating that inhibition by catechols does not occur through an unspecific mode of action. To ensure that the absence of inhibition against PHF8 was not a defect of the FDH assay, the activity of PHF8 was assayed in the presence of two known HDM inhibitors (NOG and 2,4-PDCA) and for both compounds a decreased enzyme activity was observed, thereby confirming the validity of the assay (Fig. S7).

An analysis of the mechanism of inhibition of **10** showed mixed-mode inhibition with respect to the peptide substrate (Fig. 2), as increasing inhibitor concentrations resulted in decreasing K_M values and simultaneously decreasing k_{cat} values. Meanwhile, the inhibitor exhibited non-competitive inhibition with respect to the 2OG cofactor as the effect of the inhibitor was solely to cause a lowering of k_{cat}. This demonstrates that **10** may not bind in the cofactor pocket, but could interact with the peptide substrate, either directly or indirectly.

3.4. In-cell inhibition of KDM4C by compound *10*

To evaluate the compounds in a cellular setting, the effect of **10** on the methylation level of chromatin in U2-OS cells transfected with HA-tagged full length KDM4C was studied. For reference, the known KDM4C inhibitor, 2,4-PDCA, was used to validate the assay (data not shown). 2,4-PDCA and 10 have Log*P* values in a similar range (0.59 and 0.88, respectively), and their bioavailabilities were assumed to be in a similar range as well. The compounds were shown to prevent a decrease in the H3K9 trimethylation level in cells transfected with KDM4C, as compared to untreated KDM4C transfected U2-OS cells (Fig. 3A and Fig. S9). This observation was confirmed by microscopy on immuno-stained cells (Fig. 3B and Fig. S9). Similarly, examination of the level of H3K9 mono-methylation showed that the compounds prevented an increase in signal intensity of KDM4C transfected cells treated with 2,4-PDCA and **10**. The methylation level in un-transfected cells remained unchanged with increasing concentrations of 2,4-PDCA and **10** (Fig S10). These results demonstrate that the identified compound **10** is indeed a relevant HDM inhibitor with *in cell* inhibitory effects on KDM4C.

The identified compounds have previously been associated with inhibitory effects in other cancer related studies. Compound **1**, commonly known as caffeic acid, is a known anti-oxidant, which has been shown to inhibit DNA methylation as well as growth of liver metastasis, possibly through inhibition of matrix-metalloproteases [22,23] **10** has previously been shown to inhibit cancer cell proliferation through oxidative processes [24,25]. The findings reported here indicate that the mechanism of action of the two compounds is even more complicated than initially believed, since the compounds appear to also function on the level of chromatin methylation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.03.001.

References

[1] Mosammaparast, N. and Shi, Y. (2010) Reversal of histone methylation: biochemical and molecular mechanisms of histone demethylases. Annu. Rev. Biochem. 79, 155–179.

[2] Whetstine, J.R., Nottke, A., Lan, F., Huarte, M., Smolikov, S., Chen, Z., Spooner, E., Li, E., Zhang, G., Colaiacovo, M. and Shi, Y. (2006) Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. Cell 125, 467–481.

[3] Yang, Z.Q., Imoto, I., Fukuda, Y., Pimkhaokham, A., Shimada, Y., Imamura, M., Sugano, S., Nakamura, Y. and Inazawa, J. (2000) Identification of a novel gene, GASC1, within an amplicon at 9p23-24 frequently detected in esophageal cancer cell lines. Cancer Res. 60, 4735–4739.

[4] Cloos, P.A., Christensen, J., Agger, K., Maiolica, A., Rappsilber, J., Antal, T., Hansen, K.H. and Helin, K. (2006) The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. Nature 442, 307–311.

[5] Agger, K., Cloos, P.A., Christensen, J., Pasini, D., Rose, S., Rappsilber, J., Issaeva, I., Canaani, E., Salcini, A.E. and Helin, K. (2007) UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature 449, 731–734.

[6] Tsai, M.C., Wang, J.K. and Chang, H.Y. (2010) Tumor suppression by the histone demethylase UTX. Cell Cycle 9, 2043–2044.

[7] Laumonnier, F., Holbert, S., Ronce, N., Faravelli, F., Lenzner, S., Schwartz, C.E., Lespinasse, J., Van, E.H., Lacombe, D., Goizet, C., Phan-Dinh, T.F., van, B.H., Fryns, J.P., Chelly, J., Ropers, H.H., Moraine, C., Hamel, B.C. and Briault, S. (2005) Mutations in PHF8 are associated with X linked mental retardation and cleft lip/cleft palate. J. Med. Genet. 42, 780–786.

[8] Bjorkman, M., Ostling, P., Harma, V., Virtanen, J., Mpindi, J.P., Rantala, J., Mirtti, T., Vesterinen, T., Lundin, M., Sankila, A., Rannikko, A., Kaivanto, E., Kohonen, P., Kallioniemi, O. and Nees, M. (2011) Systematic knockdown of epigenetic enzymes identifies a novel histone demethylase PHF8 overexpressed in prostate cancer with an impact on cell proliferation, migration and invasion. Oncogene.

[9] Suzuki, T. and Miyata, N. (2011) Lysine demethylases inhibitors. J. Med. Chem. 54, 8236–8250.

[10] Hamada, S., Kim, T.D., Suzuki, T., Itoh, Y., Tsumoto, H., Nakagawa, H., Janknecht, R. and Miyata, N. (2009) Synthesis and activity of N-oxalylglycine and its derivatives as Jumonji C-domain-containing histone lysine demethylase inhibitors. Bioorg. Med. Chem. Lett. 19, 2852–2855.

[11] Thalhammer, A., Mecinovic, J., Loenarz, C., Tumber, A., Rose, N.R., Heightman, T.D. and Schofield, C.J. (2011) Inhibition of the histone demethylase JMJD2E by 3-substituted pyridine 2,4-dicarboxylates. Org. Biomol. Chem. 9, 127–135.

[12] Hamada, S., Suzuki, T., Mino, K., Koseki, K., Oehme, F., Flamme, I., Ozasa, H., Itoh, Y., Ogasawara, D., Komaarashi, H., Kato, A., Tsumoto, H., Nakagawa, H., Hasegawa, M., Sasaki, R., Mizukami, T. and Miyata, N. (2010) Design, synthesis, enzyme-inhibitory activity, and effect on human cancer cells of a novel series of Jumonji domain-containing protein 2 histone demethylase inhibitors. J. Med. Chem. 53, 5629–5638.

[13] Luo, X., Liu, Y., Kubicek, S., Myllyharju, J., Tumber, A., Ng, S., Che, K.H., Podoll, J., Heightman, T.D., Oppermann, U., Schreiber, S.L. and Wang, X. (2011) A selective inhibitor and probe of the cellular functions of Jumonji C domain-containing histone demethylases. J. Am. Chem. Soc. 133, 9451–9456.

[14] Lohse, B., Nielsen, A.L., Kristensen, J.B., Helgstrand, C., Cloos, P.A., Olsen, L., Gajhede, M., Clausen, R.P. and Kristensen, J.L. (2011) Targeting histone lysine demethylases by truncating the histone 3 tail to obtain selective substrate-based inhibitors. Angew. Chem. Int. Ed. Engl. 50, 9100–9103.

[15] Sakurai, M., Rose, N.R., Schultz, L., Quinn, A.M., Jadhav, A., Ng, S.S., Oppermann, U., Schofield, C.J. and Simeonov, A. (2010) A miniaturized screen for inhibitors of Jumonji histone demethylases. Mol. Biosyst. 6, 357–364.

[16] Kawamura, A., Tumber, A., Rose, N.R., King, O.N., Daniel, M., Oppermann, U., Heightman, T.D. and Schofield, C. (2010) Development of homogeneous luminescence assays for histone demethylase catalysis and binding. Anal. Biochem. 404, 86–93.

[17] King, O.N., Li, X.S., Sakurai, M., Kawamura, A., Rose, N.R., Ng, S.S., Quinn, A.M., Rai, G., Mott, B.T., Beswick, P., Klose, R.J., Oppermann, U., Jadhav, A., Heightman, T.D., Maloney, D.J., Schofield, C.J. and Simeonov, A. (2010) Quantitative high-throughput screening identifies 8-hydroxyquinolines as cell-active histone demethylase inhibitors. PLoS One 5, e15535.

[18] Kristensen, J.B., Nielsen, A.L., Jorgensen, L., Kristensen, L.H., Helgstrand, C., Juknaite, L., Kristensen, J.L., Kastrup, J.S., Clausen, R.P., Olsen, L. and Gajhede, M. (2011) Enzyme kinetic studies of histone demethylases KDM4C and KDM6A: towards understanding selectivity of inhibitors targeting oncogenic histone demethylases. FEBS Lett. 585, 1951–1956.

[19] Couture, J.F., Collazo, E., Ortiz-Tello, P.A., Brunzelle, J.S. and Trievel, R.C. (2007) Specificity and mechanism of JMJD2A, a trimethyllysine-specific histone demethylase. Nat. Struct. Mol. Biol. 14, 689–695.

[20] Zhang, J.H., Chung, T.D. and Oldenburg, K.R. (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J. Biomol. Screen. 4, 67–73.

[21] Rose, N.R., McDonough, M.A., King, O.N., Kawamura, A. and Schofield, C.J. (2011) Inhibition of 2-oxoglutarate dependent oxygenases. Chem. Soc. Rev. 40, 4364–4397.

[22] Chung, T.W., Moon, S.K., Chang, Y.C., Ko, J.H., Lee, Y.C., Cho, G., Kim, S.H., Kim, J.G. and Kim, C.H. (2004) Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J. 18, 1670–1681.

[23] Lee, W.J. and Zhu, B.T. (2006) Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols. Carcinogenesis 27, 269–277.

[24] Babich, H., Sedletcaia, A. and Kenigsberg, B. (2002) In vitro cytotoxicity of protocatechuic acid to cultured human cells from oral tissue: involvement in oxidative stress. Pharmacol. Toxicol. 91, 245–253.

[25] Lin, H.H., Chen, J.H., Huang, C.C. and Wang, C.J. (2007) Apoptotic effect of 3,4-dihydroxybenzoic acid on human gastric carcinoma cells involving JNK/p38 MAPK signaling activation. Int. J. Cancer 120, 2306–2316.