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Inhibition of histone lysine methyltransferases G9a and GLP by ejection of structural Zn(II)

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ABSTRACT

Histone lysine methyltransferases G9a and GLP are validated targets for the development of new epigenetic drugs. Most, if not all, inhibitors of G9a and GLP target the histone substrate binding site or/and the S-adenosylmethionine cosubstrate binding site. Here, we report an alternative approach for inhibiting the methyltransferase activity of G9a and GLP. For proper folding and enzymatic activity, G9a and GLP contain structural zinc fingers, one of them being adjacent to the S-adenosylmethionine binding site. Our work demonstrates that targeting these labile zinc fingers with electrophilic small molecules results in ejection of structural zinc ions, and consequently inhibition of the methyltransferase activity. Very effective Zn(II) ejection and inhibition of G9a and GLP was observed with clinically used ebselen, disulfiram and cisplatin.

Histone posttranslational modifications (PTMs), including methylation, acetylation, phosphorylation and many others, play an important role in human gene regulation.[1] Methylation of lysine residues is catalyzed by members of histone lysine methyltransferases (KMTs) that transfer the methyl group from S-adenosylmethionine (SAM) to lysine residues on histone *N*-terminal tails, core histones and non-histone proteins. Recent structural, mutagenesis and molecular modelling studies provided basic mechanistic insight into histone methyltransferase catalysis.[2–5] SET domain-containing proteins G9a and its highly related homologue G9a-like protein (GLP) (also known as EHMT2 and EHMT1, respectively) catalyze mono-, di- and trimethylation of lysine 9 on histone 3 (H3K9me1/2/3, Fig. 1a) and several other proteins.[6] The highest methylation mark (H3K9me3) results in formation of heterochromatin, i.e. the transcriptionally inactive form of chromatin, and has been linked to the development and maintenance of various types of cancer.[7] For instance, recent work has shown that increased expression of G9a in aggressive lung cancer cells is associated with greater mortality in patients.[8] Therefore G9a and GLP have been recognized as validated targets for development of small molecule inhibitors for therapies against a variety of diseases, including cancer.[9]

Recent medicinal chemistry studies have demonstrated that inhibition of G9a and GLP (and other KMTs) can be achieved by small molecules that act as histone-competitive or/and SAM-competitive inhibitors.[10–12] BIX-01294, the first known selective inhibitor of G9a, was reported in 2007.[13] This small molecule inhibitor, which was identified by high throughput screening, targets the histone binding site. Since its discovery, the structure of BIX-01294 has been used in various structure-activity relationship (SAR) explorations and structure-based design studies, which led to the development of inhibitors with an improved potency and selectivity, and reduced toxicity to cells, such as UNC0638,[14] A-366,[15] E72,[16] and DCG066.[17] Few SAM-competitive inhibitors of G9a and GLP have also been recently reported, including BIX-01338 and BRD4770.[13,18] However, SAM-competitive inhibitors are often unselective due to high homology in SAM-binding sites between different methyltransferases. Most known inhibitors have similar inhibitory activity against G9a and GLP, and developing selective inhibitors for either one is considered challenging due to their high protein homology and similarity of the histone and SAM binding sites (~80%).[19] Nonetheless, recent work has shown that a high degree of selective inhibition can be achieved; MS012 and related structures have up to 140-fold selectivity for GLP over G9a.[20]

In order to establish proper folding and enzymatic activity, G9a and GLP methyltransferases each contain four structural zinc ions. Both enzymes contain two distinguishable types of zinc fingers; three Zn(II) ions are chelated in a triangular cluster by 9 cysteines (Fig. 1b, top left), whereas one Zn(II) ion is chelated by 4 cysteines in a Cys_4-type zinc finger (Fig. 1b, top right).[21] The latter zinc finger is adjacent to the SAM-binding site. Recent studies have highlighted that significant efforts have been made in developing

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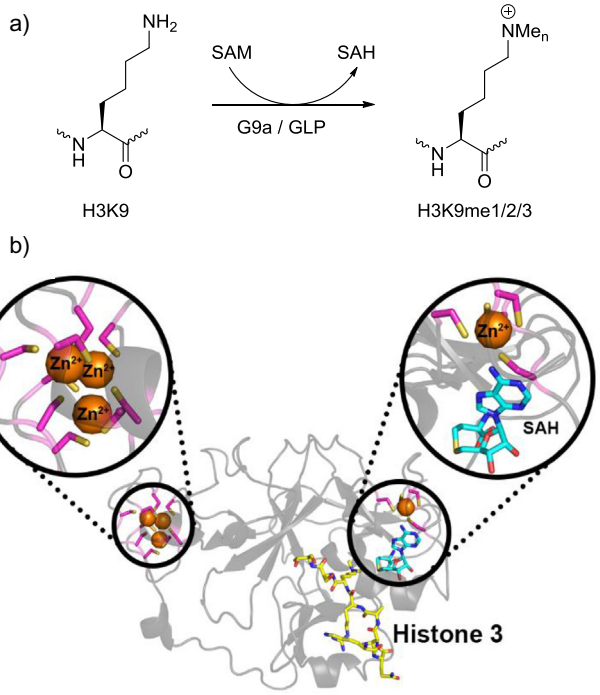


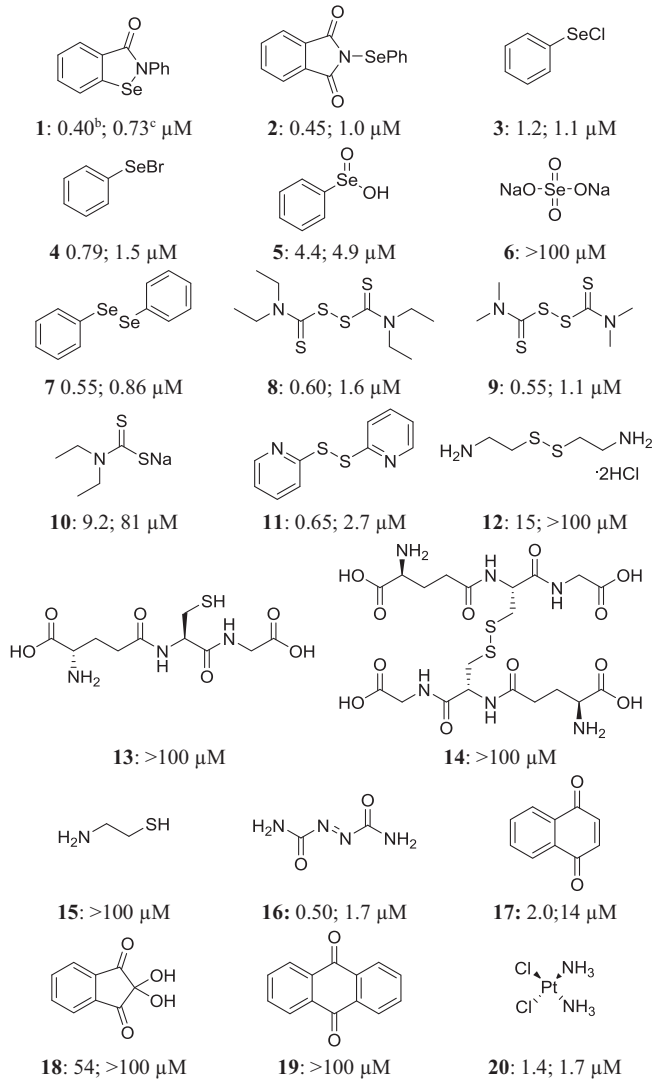
Fig. 1. a) G9a- and GLP-catalyzed methylation of H3K9 (n = 1, 2, 3); b) Crystal structure of GLP in complex with histone 3 peptide (yellow) and S-adenosylhomocysteine (SAH, light cyan); a zoomed view on the Cys residues (magenta) involved in the chelation of structural Zn(II) (orange) (PDB ID: 2RFI).

strategies for targeting labile Zn-fingers with electrophilic small molecules, most notably by ebselen and disulfiram.[22,23] Inhibition of important biological processes by the release of structural zinc ions has been shown for a variety of proteins, including nucleocapsid 7,[24] p300,[25] γ-butyrobetaine,[26] and histone lysine demethylase JMJD2A.[27] We hypothesized that it would be possible to inhibit G9a and GLP methyltransferases by small molecule-mediated ejection of structural zinc ions. It was envisioned that the ejection of Zn(II) from the Cys_4-Zn finger, which is located adjacent to the SAM-binding site (Fig. 1b, top right), would lead to a loss of the methyltransferase activity of G9a and GLP.

We initiated our investigations by testing whether 20 known and potential zinc ejectors, including clinically used ebselen, disulfiram and cisplatin, have an ability to inhibit the G9a and GLP methyltransferase activity (Table 1). The chosen examples include: selenium-based compounds **1**–**7**, sulfur-based compounds **8**–**15**, and various other potential Zn(II) ejectors **16**–**20**. Initially, all compounds were tested at a concentration of 10 μM against both G9a and GLP. Therefore, methylation of a synthetic 15-mer peptide (residues 1–15) mimic of the *N*-terminal histone 3 tail containing a lysine at position 9 (H3K9) was monitored using matrix assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry.[28] Representative inhibition data for GLP-catalyzed methylation of H3K9 can be found in Fig. 2. Molecules that did not show significant inhibition at 10 μM, were also tested at 100 μM. Inhibition data at 10 and 100 μM for all other compounds can be found in Figs. S1–S4. For those compounds that showed >50% inhibition at a concentration of 100 μM, half maximum inhibitory concentrations (IC_{50}) were obtained using a MALDI-TOF based assay using 200 nM enzyme concentrations (Table 1 and Figs. S1–S32).

In the absence of inhibitor, 15-mer H3K9 histone mimic underwent near quantitative trimethylation (m/z = 1603.1 Da, Fig. 2a); this result is consistent with our recent studies on KMT-catalyzed methylation of lysine.[29] For ebselen **1**, which is known to inhibit

Table 1
Inhibition IC_{50} data.[a]



[a]Half maximum inhibitory concentration (IC_{50}) obtained at 200 nM enzyme concentration.
[b]IC_{50} for G9a.
[c]IC_{50} for GLP.

various zinc finger containing proteins, such as metallothionein,[30] histone lysine demethylase JMJD2A,[27] and γ-butyrobetaine hydroxylase,[26] submicromolar IC_{50} values were obtained (0.40 μM for G9a and 0.73 μM for GLP), demonstrating very effective inhibition. Notably, at 10 μM concentration of ebselen, only unmethylated peptide was observed in MALDI-TOF spectrum (Fig. 2b, m/z = 1560.9). Related seleno compounds **2**–**5** were also observed to be excellent inhibitors of G9a and GLP; IC_{50} values were found to be 0.45–4.4 μM for G9a, and 1.0–4.9 μM for GLP. Sodium selenate **6** only showed ~10% inhibition for G9a and GLP at 100 μM concentration (Figs. S2 and S4). Diphenyl diselenide **7** inhibited G9a and GLP with IC_{50} = 0.55 μM and 0.86 μM, respectively.

Having shown that selenium-based compounds act as good inhibitors of G9a and GLP, we examined related sulfur-based small molecules as potential inhibitors for these two methyltransferases. Dithiocarbamates disulfiram **8** (Fig. 2c) and thiram **9** inhibited the activity of G9a with submicromolar IC_{50} values of 0.60 and