Synthesis and Structure—Activity Relationship Investigation of Adenosine-Containing Inhibitors of Histone Methyltransferase DOT1L

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Supporting Information

ABSTRACT: Histone3-lysine79 (H3K79) methyltransferase DOT1L has been found to be a drug target for acute leukemia with MLL (mixed lineage leukemia) gene translocations. A total of 55 adenosine-containing compounds were designed and synthesized, among which several potent DOT1L inhibitors were identified with Ki values as low as 0.5 nM. These compounds also show high selectivity (>4500-fold) over three other histone methyltransferases. Structure—activity relationships (SAR) of these compounds for their inhibitory activities against DOT1L are discussed. Potent DOT1L inhibitors exhibit selective activity against the proliferation of MLL-translocated leukemia cell lines MV4;11 and THP1 with EC50 values of 4−11 μM. Isothermal titration calorimetry studies showed that two representative inhibitors bind with a high affinity to the DOT1L:nucleosome complex and only compete with the enzyme cofactor SAM (S-adenosyl-L-methionine) but not the substrate nucleosome.

INTRODUCTION

Post-translational modifications of a lysine residue of histone play important roles in gene regulation.1 These chemical modifications include acetylation and methylation, which change the electrostatic and/or steric properties of histone and thereby control the accessibility of genes wrapped around the histone core. Histone methyltransferase (HMT) consists of a family of >50 enzymes that methylate the side chain amino (or guanidine) group of a lysine (or arginine) residue of histone or other proteins.2,3 As schematically illustrated in Figure 1, all HMTs use S-adenosyl-L-methionine (SAM) as the methyl donor (enzyme cofactor), producing methylated substrates as well as S-adenosyl-L-homocysteine (SAH). Aberrant histone methylations have been linked to many types of cancer,4 and there is therefore great interest in developing inhibitors of these enzymes.5 However, only a limited number of compounds have been reported to be HMT inhibitors.5−8

DOT1L is a histone lysine methyltransferase that specifically methylates the residue Lys79 of histone 3 (H3K79).9,10 The catalytic domain of human DOT1L is highly conserved from yeasts to mammals. There are two features that make DOT1L a unique HMT. First, H3K79 is located in the ordered histone octamer core structure, while the methylation sites of all other HMTs are in the unordered tail of a histone protein. Second, DOT1L is the only histone lysine methyltransferase that belongs to class I methyltransferases. All other histone lysine methyltransferases are class V methyltransferases with a conserved SET domain. Of particular interest is that DOT1L has been proposed to be a target for acute leukemia with MLL (mixed lineage leukemia) gene translocations.11−13 As

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compared to other leukemias, this subtype of leukemia has a poor prognosis with a 5 year survival of <40%. Studies showed that several predominant fusion partners of onco-MLL genes, including AF4, AF9, AF10, and ENU, can recruit DOT1L, which methylates H3K79, and causes overexpression of leukemia relevant genes (such as Hoxa9 and Meis1) that eventually leads to leukemia initiation. In addition, H3K79 hypermethylation was also found to be a hallmark of MLL-rearranged leukemia. A potent inhibitor EPZ004777 (1, Figure 1) was recently reported, which can selectively inhibit the proliferation of MLL-rearranged leukemia cell lines and prolong the life spans of experimental animals in a mouse model of human MLL leukemia. This further pharmacologically validated DOT1L as an antileukemia target. However, because of its very short half-life in plasma, 1 has a low plasma concentration of ~0.5 μM even when supplied continuously with an implanted pump. Clearly, more stable inhibitors are needed.

Our early work also disclosed a structure-based approach to the identification of several potent DOT1L inhibitors, such as 2–4 (Figure 1), showing that introduction of an N⁶-substituent on the adenine ring can provide high selectivity for DOT1L. Likely due to containing a reactive N-2-iodoethyl group (a precursor of aziridinium), these DOT1L selective inhibitors do not have specific activity in killing MLL-rearranged leukemia cells. In a parallel effort to find competitive DOT1L inhibitors, we also identified urea-containing compounds that possess strong inhibitory activity against DOT1L. Here, we report the synthesis, structure–activity relationships (SAR), and biological activities of 55 compounds targeting DOT1L. In addition, isothermal titration calorimetry (ITC) was used to investigate the binding affinities of selected inhibitors to DOT1L.

## RESULTS AND DISCUSSION

### Inhibitor Design and SARs for DOT1L Inhibition

The poor pharmacokinetics of 1 is likely due to its 7-deaza-adenosine moiety, which can be recognized by many human enzymes, such as adenosine deaminase, hydrolase, or nucleosidase, leading to a rapid degradation. Other compounds containing adenosine or an adenosine-like group suffer from a similar metabolic instability. In our early work, we found that DOT1L, but not other HMTs, can well tolerate an N⁶-substituent on the adenine ring. Although the amino acid group forms an H-bond with the adenine moiety (at physiological pH) of compound 1, it does not have specific activity in killing MLL-rearranged leukemia cells. To address this, we identified urea-containing compounds that possess strong inhibitory activity against DOT1L. Here, we report the synthesis, structure–activity relationships (SAR), and biological activities of 55 compounds targeting DOT1L. In addition, isothermal titration calorimetry (ITC) was used to investigate the binding affinities of selected inhibitors to DOT1L.

First, we synthesized a series of SAH derivatives 2 and 5−12 containing nine N⁶-substituents. The structures and inhibitory activities of these compounds are shown in Table 1, together with those of SAH, the common product of HMT-catalyzed reactions and a nonselective HMT inhibitor. Compound 2 with an N⁶-methyl is still a potent DOT1L inhibitor with a Ki value of 290 nM. Although it is slightly less active than SAH (Ki = 160 nM), its methyl group provides excellent selectivity for DOT1L, with only weak or no activity against other HMTs. Compounds 5–8 with an allyl, cyclopropyl, isopropyl, and n-butyl group, respectively, have considerably reduced activity with Ki values of 2.0–3.8 μM, as compared to 2. Compounds 9–11 bearing a branched butyl or pentyl chain are even less active (Ki = 6.5–8.9 μM). In addition, compound 12 having an N⁶-benzyl group has the second best inhibitory activity in this series with a Ki of 1.1 μM. These results show that the small methyl group in 2 provides the best activity as well as selectivity.

Next, we wanted to replace the highly polar, charged amino acid moiety (at physiological pH) of compound 2 and SAH, since it renders these compounds limited bioavailability. Although the amino acid group forms five H-bonds with DOT1L, the pocket also contains several hydrophobic residues including Phe239, Val169, Thr139, and Tyr 136 (Figure 2), suggesting that a more lipophilic side chain might also be favorable. To this end, 15 compounds with a general structure in Chart 1 were synthesized and tested for their inhibition against DOT1L, and the results are also shown in Chart 1. In general, these compounds are inactive or only weakly active. Compound 13 (Ki = ~100 μM) is deacetyl-SAH, but it is >600x less active than SAH. Compound 14 with a shorter linker has a Ki value of 25 μM, being slightly more active than 13. Changing −S− to −NH− resulted in an inactive compound 15. Compounds 16–18 having a carboxyl, hydroxyl, or propargyl group possess no activity against DOT1L. Compounds 19–25 containing a phenyl or hetercyclic aromatic ring are also inactive. However, compounds 26 and 27 with a carbamate group, which are actually the precursors for making

![Figure 2. Active site of the DOT1L2 structure (PDB code: 3SR4), with compound 2 shown as a ball and stick model and hydrogen bonds as dotted lines.](image-url)

| Table 1. Structures of Inhibitors and Their Ki Values against DOT1L |
|------------------------|------------------|-----------------|
| Compd | R− | Ki (μM) |
| SAH | −H | 0.16 |
| 2 | −CH3 | 0.29 |
| 5 | −allyl | 2.0 |
| 6 | −i-Pr | 2.0 |
| 7 | −i-Pr | 3.8 |
| 8 | −n-Bu | 2.8 |
| 9 | −C(CH3)3 | 7.8 |
| 10 | −CH2CH2CH(CH3)2 | 6.5 |
| 11 | −CH2C(CH3)3 | 8.9 |
| 12 | −CH2Ph | 1.1 |

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compounds 13 and 15, were found to be weak inhibitors of DOT1L with $K_i$ values of 50 and 46 μM. These two compounds are of interest, since they possess greatly improved lipophilicity as well as unexpected, higher activity than 13 and 15.

To find compounds with improved potency, three related series of compounds based on the structures of 26 and 27 were synthesized, including carbamates, amides, and ureas, and the results are summarized in Table 2. Compound 28, a thioether analogue of 27, possesses essentially the same activity ($K_i$ = 47 μM), while changing the benzyl group to an ethyl for compound 29 makes it inactive, showing that an aromatic ring is favored for the R2 group (in the general structure shown in Table 2). Introducing an additional isopropyl substituent into the S'-N atom for compound 30 ($K_i$ = 22 μM) resulted in a ~2-fold activity increase, as compared to its parent compound 27. We next sought whether other functional groups can provide improved inhibitory potency. Analogous amide compounds 31−33 were found to be inactive. A reversed amide compound 34 (with the same skeleton length) as well as a reversed carbamate compound 35, however, exhibit ~2× improved inhibition against DOT1L with $K_i$ values of 22 and 16 μM, as compared to 26−28. Urea compound 36 was found to have a greatly enhanced activity with a $K_i$ value of 1.9 μM. Compounds 37−40 containing a different R2 group were therefore synthesized to optimize this position. Although 37−39 having 2-furanyl-methyl, propyl, and cyclohexyl group are less active than 36, compound 40 with a $K_i$ of 0.55 μM exhibits ~3× more activity, showing that a phenyl group here is more favorable than an alkyl or aryl-substituted alkyl group. In addition, these results demonstrate that both −NH− moieties of the urea group are very important, with each one offering ~25 to >50-fold activity improvements, as compared to less favored −O− and −CH2− groups (e.g., compound 36 vs 28, as well as compound 40 vs 34/35). These two −NH− could serve as H-bond donors to increase the binding affinity to DOT1L. Next, compounds 41 and 42 having 2- and 4-methylene linkers, respectively, were prepared and found to possess less activity ($K_i$ = 18 and 1.1 μM) than compound 40 with a 3-methylene linker. This shows that a three −CH2− linker between the urea and the S'-position represents the optimal length for the inhibition.

Compounds 43−54 that are derivatives of 40 were synthesized in an effort to find an appropriate substituent on the phenyl ring. As can be seen in Table 2, all of the 4-substituents in 43−49 ($K_i$ = 58−350 nM), including isopropyl, tert-butyl, −Cl, −Br, −I, −CF3, and −OCF3, provide improved activity, as compared to the parent compound 40 ($K_i$ = 550 nM). Among these, bulky tert-butyl (in 44) and electron-withdrawing −CF3 group (in 48) render ~8-fold potency enhancement. For halogen substituents (in compounds 45−47), a trend of −Cl > −Br > −I was observed. Compound 50 with a 2-ethyl substituent possesses a slightly decreased activity ($K_i$ = 690 nM), while 51 having a 3-ethyl exhibits a 4× stronger
inhibition ($K_i = 130 \text{ nM}$). 2,4-Difluoro-substituted compound S2 is less active than S0, while another substituted compound S3 with 3,5-di-Cl-C$_6$-F represents one of the best inhibitors with a $K_i$ value of 80 nM. Compound S4 with a 1-naphthyl R$_2$ group (i.e., 2,3-disubstituted phenyl) is considerably less active ($K_i = 2700 \text{ nM}$) than compound S0.

We next synthesized compound S55 by replacing the $-S-$ of S4 with a $-\text{N}(p$-Pr$)-$ linkage, which in compound S30 shows improved activity. Compound S55 was found to be an extremely potent DOT1L inhibitor with a $K_i$ value of 0.46 nM. The large activity boost (as compared to that of S4 with a $K_i$ of 70 nM) might be due to coordinated protein conformational changes induced by cobinding of the $N$(4-tet-butylphenyl)urea side chain and the $-\text{N}(p$-Pr$)-$ group of S55. Introducing a N$_2$-methyl group led to compound S56, which possesses a similar inhibitory activity against DOT1L ($K_i = 0.76 \text{ nM}$), while compounds S57 and S58 having N$_2$-allyl and benzyl groups were found to be less active with $K_i$ values of 12 and 22 nM, respectively.

Synthesis and activity testing of compounds 2 and 5–58 targeting DOT1L provide several novel observations for SAR. First, a series of urea-containing adenosine derivatives were found to be potent DOT1L inhibitors with $K_i$ values as low as 0.46 nM, with each of the two $-\text{N}(p$-Pr$)-$ moieties of the urea offering 25- to >50-fold activity enhancements. Second, 3- or 4- substituted phenyl is a preferred R$_2$ group. Third, introducing a linker between the urea and the 5′-position, such as an isopropyl, can further improve the inhibitory activity. Fourth, a 3-methylene linker between the urea and the 5′-position is the optimal length for DOT1L inhibition. Fifth, an N$_2$-substituent on the adenine ring in general decreases the activity, while a small substituent at this position, such as methyl (as found in compound S6 with a $K_i$ of 0.76 nM), could help provide desired selectivity as well as stability, without much activity loss.

**Biological Activity of Selected DOT1L Inhibitors.** From the above SAR studies, we have obtained several highly potent DOT1L inhibitors having $K_i$ values as low as 0.46 nM. We next investigated whether these compounds have enzyme activity against other HMTs. Selectivity is of importance for developing clinically useful DOT1L inhibitors that target the SAM binding site, since all HMTs use a similar mechanism of catalysis. Our previous structure-guided design showed a N$_2$-substituent, such as the methyl in compound 2, provides excellent selectivity for DOT1L (Table 3). Potent DOT1L inhibitors S55–S58 were chosen to be tested against three representative HMTs, that is, PRMT1, CARM1 (or called PRMT4), and SUV39H1. PRMT1 and CARM1 are histone/protein arginine methyltransferases, which also belong to class I methyltransferases and share certain similar structural features as DOT1L. SUV39H1 is a typical SET domain histone lysine methyltransferase, belonging to the class V methyltransferases having a distinct structure. As can be seen in Table 3, drastically different from nonselектив inhibitor SAH, compounds S5–S8 exhibit essentially no activity against all of these three HMTs, showing excellent selectivity (>4500-fold).

Next, we tested the activities of compounds S5-S8 against the proliferation of three human leukemia cell lines, MV4;11, THP1, and NB4. MV4;11 and THP1 are MLL gene translocated leukemia cells, harboring MLL-AF4 and MLL-AF9 oncoproteins, respectively, while NB4 possesses a wild-type MLL. Figure 3 shows time-dependent activities of two representative compounds S5 and S6 on MV4;11 cells at four different concentrations (i.e., 1, 3, 10, and 30 μM). These two potent DOT1L inhibitors work very slowly in blocking the proliferation of the MLL-translocated cells. For example, at 10 μM, they had negligible activity against the cell growth even on day 10. However, S5 and S6 (at 10 μM) were able to exhibit 61.7 and 43.4% cell growth inhibition on day 15 as well as more pronounced activities (91.7 and 77.6% inhibition) on day 20. Other doses of these two compounds showed a similar tendency of activity. In addition, as shown in Table 4, compounds S5 and S6 exhibit good cell growth inhibition against MLL-translocated cells MV4;11 and THP1 with EC$_{50}$ values of 4.4–11.0 μM but are almost devoid of activity (EC$_{50}$ > 77 μM) in a 20 day treatment against NB4 leukemia cells bearing a wild-type MLL gene. This indicates these two DOT1L inhibitors act in a mechanism drastically different from that of traditional chemotherapeutic agents, such as cisplatin, which is able to kill all of these leukemia cells within a short period of time (standard 2 day incubation) with EC$_{50}$ values of 0.59–3.0 μM (Table 4). Previous biological studies$^{2a}$ demonstrate that the slow action of DOT1L inhibitors is likely due to a relatively long time required for cellular events that lead to cell growth inhibition, including blocked H3K79 methylation, followed by decreased levels of mRNA expression for methyl-H3K79 targeted genes, as well as ultimately the depletion of the gene products (proteins) key to the cell proliferation. As can be seen in Table 4, two other potent DOT1L inhibitors S7 and S8 also exhibit good activity against MV4;11 and THP1 with EC$_{50}$ values of 5.9 – 11.2 μM, although they (especially for S8) also have activity on NB4, showing a decreased selectivity.

**Molecular Modeling and ITC Studies.** Our early X-ray crystallographic study revealed that the binding site of the N$_2$ substituent is actually a mainly hydrophobic pocket that directly exposes to the solvent (Figure 2).$^{7b}$ This could explain that although this group provides high selectivity for DOT1L, it does not increase the binding affinity (Table 1). However, the big, hydrophobic, urea-containing side chains render both greatly enhanced activity and selectivity. The next question of interest is where these urea side chains bind in DOT1L. X-ray crystallography is the method of choice to determine this. However, we have not obtained quality single crystals of DOT1L in complex with any one of these urea-containing inhibitors. We explored molecular modeling (docking) to predict the binding site of these groups, using the crystal structure of the DOT1L:SAM complex$^{10}$ as the docking template. As representative shown in Figure S1 in the Supporting Information for compound 36, all of these urea-containing groups are invariably predicted by the docking program Glide$^{17}$ in Schrödinger$^{18}$ (version: 2010) to extend into the nucleosome (substrate) binding pocket of DOT1L through the "lysine binding channel", which holds the tightly clustered $-\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{H}_2\text{N}^+$- linkers of 10 docking structures of 36 (Figure S1a in the Supporting Information). The bulky and

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Table 3. $K_i$ Values (μM) of DOT1L Inhibitors on Other HMTs

<table>
<thead>
<tr>
<th>Compd</th>
<th>DOT1L</th>
<th>PRMT1</th>
<th>CARM1</th>
<th>SUV39H1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAH</td>
<td>0.16</td>
<td>0.40</td>
<td>0.86</td>
<td>4.9</td>
</tr>
<tr>
<td>2</td>
<td>0.29</td>
<td>22.7</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>S5</td>
<td>0.00046</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>S6</td>
<td>0.00076</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>S7</td>
<td>0.012</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>S8</td>
<td>0.022</td>
<td>&gt;100</td>
<td>&gt;100</td>
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</table>
lipophilic aromatic group is predicted to be located in a mainly hydrophobic pocket on the nucleosome binding surface and the urea moiety to form a H-bond with the protein (Figure S1b in the Supporting Information). These results seem to be reasonable, since the amino acid binding pocket in the DOT1L:SAM structures has no room to hold such long and bulky side chains.

The docking studies, if real, indicate that these urea-containing compounds are bisubstrate type inhibitors, which occupy the binding sites of SAM and nucleosome. These inhibitors should therefore compete with both SAM and the substrate nucleosome. Structurally similar inhibitor 1 was reported to be competitive with SAM. A kinetic study from the same group also showed the $K_m$ value for nucleosome is 8.6 nM but did not report whether 1 competes with nucleosome on binding to DOT1L. It is known that the binding of nucleosome to DOT1L involves very large surface areas including those from histone proteins (H3, H4, H2A, and H2B) and the DNA around the histone core. However, the $K_d$ (dissociation constant) value of nucleosome has not been measured.

We used ITC to determine the binding affinities of inhibitors 55, 56, SAH, and the substrate nucleosome. First, each of these compounds was titrated individually into DOT1L in a 50 mM HEPES buffer (pH 7.0) with 150 mM NaCl. As summarized in Table 5 and representatively shown in Figure 4, the $K_d$ values for SAH, 55, 56, and nucleosome were determined to be 360, 170, 140, and 14 nM, respectively. The high-affinity binding of nucleosome to DOT1L ($K_d = 14$ nM) is consistent with their large surface interactions as well as the reported $K_m$ value. The binding affinity of SAH is also comparable to its $K_i$ value (160 nM, Table 1) as determined by the enzyme inhibition assay. However, the measured $K_d$ values of 55 and 56 are >200× larger than their $K_i$ values of 0.46 and 0.76 nM. Next, we investigated the binding affinities of these ligands to the DOT1L:nucleosome (1:1 molar ratio, 5 μM) complex, which better mimics the enzyme inhibition assay given the tight binding of nucleosome to DOT1L. Compounds SAH, 55, and 56 were found to have considerably higher affinities to the DOT1L:nucleosome complex with $K_d$ values of 150, 66, and 86
The enhanced affinity for SAH can be conceivable since SAH and nucleosome have different binding sites in DOT1L. However, for compounds 55 and 56, these experimental results are inconsistent with our docking studies, which suggest that these two molecules are competitive with nucleosome and (if real) should exhibit greatly reduced binding affinities to DOT1L in the presence of 5 μM nucleosome (≫Kd of 14 nM). The ITC experiments therefore showed that these two inhibitors do not compete with nucleosome. One possibility is due to the flexibility of DOT1L, which could render appropriate conformational changes at the active site of DOT1L upon the binding of inhibitor 55/56. Such an induced fit could allow cobinding of 55 (or 56) and nucleosome. In addition, we used ITC to determine whether 55 is competitive with SAH. Compound 55 was titrated into the DOT1L:nucleosome complex in the presence of increasing concentrations of SAH. As shown in Table S5 and Figure S2 in the Supporting Information, the Kd values of 55 measured in the presence of 1, 2.5, 5, 10, and 20 μM SAH increase in a linear fashion (r² = 0.97), showing a competitive mode of action.

The large difference between the Kd and Ki values of 55 and 56 measured by ITC and enzyme inhibition assay, respectively, could also be due to the protein conformational changes induced by these ligands. The rapidly formed initial EI (enzyme:inhibitor) complex could undergo a time-dependent conversion to a more tightly bound complex EI*.

Chemistry. The general methods for synthesizing compounds 2 and 5–58 are shown in Scheme 1. N6-substituted adenosine 59 was prepared from inosine by triacetate protection, conversion to 6-chloro by treatment with SOCl2, deprotection, and heating with a primary amine. Compounds 2 and 5–12 were synthesized from 59 according to our previous method. Other 5′-S-containing compounds 26, 28, 29, and 31–54 were prepared from 2′,3′-acetonide-protected adenosine by a Mitsunobu reaction with thiaoic acid, followed by
in situ hydrolysis of the thioester product with NaOMe and a one-pot alkylation with a bromide in MeOH. A Mitsunobu reaction of 2,3′-acetonide-protected adenosine with phthalamide, followed by NH₂NH₂·H₂O treatment, gives rise to adenosine derivative 61 having a S′-NH₂, which was alkylated with Z-protected 3-iodopropylamine to give compound 27. To make N-isopropyl-containing compounds 55–58, compound 61 was subjected successively to a reductive amination with acetonitrile and NaN₃BH₃ to add an N-isopropyl group, a Michael addition with methyl acrylate, and a reduction with LiAlH₄ to give compound 62 with a terminal —OH. The —OH was then converted, by a Mitsunobu reaction followed by NH₂NH₂·H₂O treatment, to a —NH₂, which was further reacted with an isocyanate, affording, after deprotection of 2,3′-acetonide, compounds 55–58.

■ CONCLUSION

This work provides the synthesis, SAR, and ITC studies of a series of inhibitors of human HMT DOT1L, a novel target for acute leukemia with MLL gene translocations. First, a total of 55 adenosine-containing compounds were designed and synthesized, among which several highly potent DOT1L inhibitors were identified with Kᵢ values as low as 0.5 nM. Second, SAR analysis of these compounds shows that (1) replacing the amino acid moiety of SAH with an N-phenyl-substituted urea functional group leads to a series of potent and selective DOT1L inhibitors; (2) replacing the −S− as found in SAH to an −N(isopropyl)− group offers additional activity enhancement; (3) the optimal linker between the urea and the S′-groups is −CH₂CH₂CH₂−; and (4) a small substituent (e.g., methyl) at the N⁰-position of adenine ring renders high selectivity without much activity loss. Third, several representative DOT1L inhibitors demonstrate selective activity against the proliferation of MLL-rearranged leukemia cells with the EC₅₀ values of 4–11 μM. However, it takes a relatively long time (>10 days) for these compounds to exert growth arrest, showing a different mechanism of action from traditional chemotherapeutic drugs. Finally, ITC experiments showed urea-containing inhibitors 55 and 56 are able to bind with a high affinity (Kᵢ: 66 and 86 nM) to the DOT1L:nucleosome complex, and only compete with SAM/SAH, but not the substrate nucleosome, on binding to DOT1L.

■ EXPERIMENTAL SECTION

All reagents were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich (Milwaukee, WI). Compounds were characterized by ¹H NMR on a Varian (Palo Alto, CA) 400 MR spectrometer. The purities of all compounds were determined by a Shimadzu Prominence HPLC with a Zorbax C18 or C8 column (4.6 mm × 250 mm) monitored by UV absorbance at 254 nm or ¹H (at 400 MHz) absolute spin-count quantitative NMR analysis using imidazole as an internal standard. The purities of all compounds were found to be >95%. The synthesis and characterization of compounds 5–58 can be found in Experimental Section in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

Figures S1 and S2 and Experimental Section showing docking results, linear correlation of Kᵢ values of 55 with the competing SAH concentrations, and detailed compound synthesis and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.
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ABBREVIATIONS USED
HMT, histone methyltransferases; H3K79, histone3-lysine79; ITC, isothermal titration calorimetry; MLL, mixed lineage leukemia; SAM, S-adenosyl-l-methionine; SAH, S-adenosyl-l-homocysteine; SAR, structure–activity relationship

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