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Using 'biased-privileged' scaffolds to identify lysine methyltransferase inhibitors

Sudhir Kashyap [a,b,*], Joel Sandler [a], Ulf Peters [a], Eduardo J. Martinez [a], Tarun M. Kapoor [a,*]

[a] Laboratory of Chemistry and Cell Biology, Rockefeller University, New York, NY 10065, USA
[b] Organic & Biomolecular Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, AP, India

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ABSTRACT

Methylation of histones by lysine methyltransferases (KMTases) plays important roles in regulating chromatin function. It is also now clear that improper KMTases activity is linked to human diseases, such as cancer. We report an approach that employs drug-like 'privileged' scaffolds biased with motifs present in *S*-adenosyl methionine, the cofactor used by KMTases, to efficiently generate inhibitors for Set7, a biochemically well-characterized KMTase. Setin-1, the most potent inhibitor of Set7 we have developed also inhibits the KMTase G9a. Together these data suggest that these inhibitors should provide good starting points to generate useful probes for KMTase biology and guide the design of KMTase inhibitors with drug-like properties.

1. Introduction

Covalent and reversible post-translational modifications (PTMs) of histones, proteins that assemble into octamers around which DNA is 'spooled', play key roles in regulating gene expression in eukaryotes.[1–3] The histone-based structures, called nucleosomes, are the basic building blocks of chromatin.[4] In current models, PTMs of histone, such as acetylation, methylation, phosphorylation, glycosylation, sumoylation or ubiquitination, modulate protein recruitment to nucleosomes and can regulate chromatin organization.[5–8] For example, methylation of lysine-4 at the N-terminus of histone H3 recruits different proteins to chromatin and is believed to 'mark' a transcriptionally active 'on'-state of chromatin.[9,10] Like other dynamic PTMs, the level of histone lysine methylation is regulated by a balance in the activity of lysine methyltransferases (KMTases), which add the methyl group, and demethylases, which reverse the modification.[11] While important advances have been made in identifying KMTases, our understanding of the dynamic regulation and function of the different histone methylations remains incomplete. In line with critical roles of these enzymes in basic cellular processes, their dysfunction has been linked to human diseases, such as cancer.[12] Therefore, developing small molecule inhibitors of KMTases to probe their functions and to properly validate these enzymes as targets for chemotherapy has become an important goal.

It has been estimated that there are over 50 lysine methyltransferases in humans.[13,14] Currently, selective inhibitors for a handful of KMTases (e.g., G9a and Dot1L) have been reported.[15–17] However, when compared to other important enzyme targets (e.g., kinases),[18] the chemical diversity of available KMTase inhibitors and structural information on the modes of inhibition is restricted to a few examples. Therefore, designing probes for different KMTases remains challenging.

KMTases use *S*-adenosyl methionine (SAM) as the source of the methyl group in the reaction they catalyze (Fig. 1A). Structural analyses of KMTases have provided insight into how these enzymes bind this cofactor.[19,20] Unlike kinases, in which the ATP's phosphate groups are polar and the key hydrophobic contacts are made with the adenine, KMTases make numerous contacts with most of the atoms in SAM. Consistent with these observations SAM-related compounds, such as sinefungin and *S*-adenosylhomocysteine, inhibit KMTases and have been useful in studies analyzing their activities (Fig. 1B).[21–25] In addition, systematic modifications of SAM have led to the development of inhibitors of KMTases.[16,17] Encouraged by these findings, we developed a strategy that uses features of SAM to develop drug-like inhibitors for KMTases. We reasoned that 'privileged' chemical scaffolds, which have provided good starting points for developing inhibitors for different enzymes (e.g., kinases and myosins),[26,27] may be

* Corresponding authors. Tel.: +91 402 716 1649; fax: +91 402 716 0387 (S.K.); tel.: +1 212 327 8173; fax: +1 212 327 7358 (T.M.K.).
E-mail addresses: skashyap@iict.res.in (S. Kashyap), kapoor@rockefeller.edu (T.M. Kapoor).

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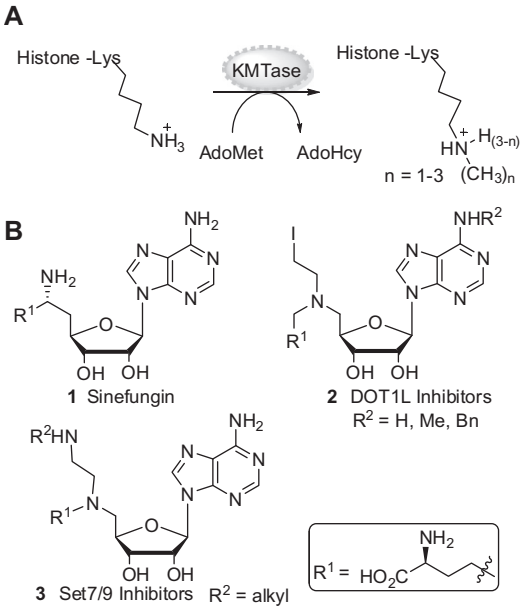

Figure 1. (A) Schematic for the KMTase catalyzed reaction. (B) SAM-based inhibitors of KMTases.

coupled to homocysteine to yield broad specificity KMTase inhibitors (Fig. 2A). In particular, the heterocycles (e.g., diaminopyrimidine and indoles) common in many kinase inhibitors could mimic the adenosine and a benzyl linkage to the homocysteine could position the key functional groups in correct spacing and orientations. Here we report the design, development and validation of SETin-1, an inhibitor of histone KMTases based on 'biased-privileged' scaffolds.

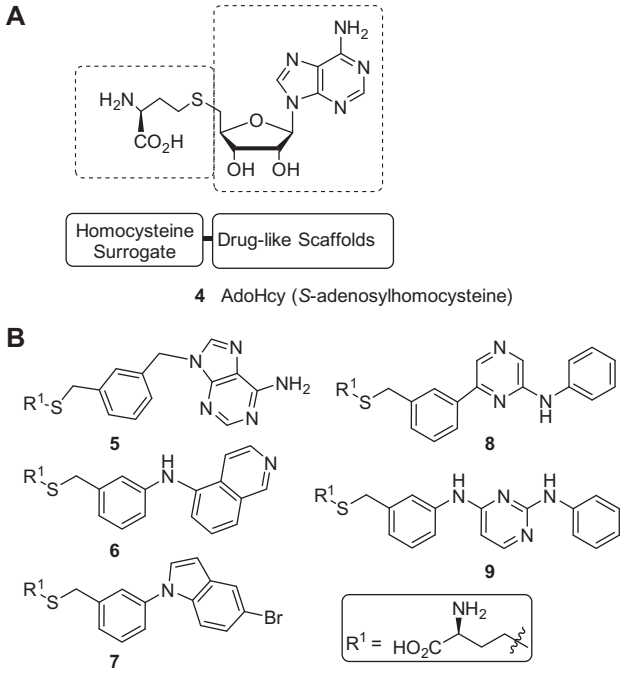

Figure 2. (A) Schematic for the 'biased-privileged' scaffold based strategy to develop KMTase inhibitors. (B) Compounds designed and tested.

2. Results and discussion

We generated a handful of compounds in which homocysteine was appended to 'privileged' chemical scaffolds, which are commonly found in drugs (e.g., kinase inhibitors) (Fig. 2B). Briefly, N-alkylation, palladium-catalyzed Suzuki coupling and copper catalyzed Buchwald couplings were used to obtain compounds **5**–**9**.[28]

We tested their activities against the KMTase, SET7, a well-studied member of this enzyme family. Recombinant SET7 (residues 52–366) was expressed in bacteria as a GST-fusion and purified as previously described.[20,29] Compounds were tested using an ELISA assay in which biotin-conjugated histone H3 peptide (H31-20-cys-biotin) was immobilized on multi-well plates and methylation detected using an antibody that recognizes the monomethylated lysine-4 of histone H3. This assay was based on the method reported by Kubicek et al.[15] Compound **7** was the only active compound, revealing modest activity (~12% inhibition at 40 μM), while all others tested were inactive (Fig. 3). Encouraged by these data, we generated analogs of **7**.

As a first step, we focused on the homocysteine portion of compound **7**, with the goal to reduce the α-amino acid character. We generated γ-butyric acid and meta-benzoic acid substituted versions of **7** using a Buchwald coupling and substitution reactions (Scheme 1A). We found that compounds **10** and **11** were more potent inhibitors of SET7 than **7**. As compound **11** was the most potent, we retained the *meta*-benzoic acid moiety for subsequent analysis. We next examined how changes in the substitutions of the indole impacted activity. We found that Br-substitution at the 4-position (**12**) of the indole increased potency and replacing the Br with a phenyl group (**13**) was even more effective (Scheme 1B). We then examined whether the thioether functionality could be replaced by a secondary amine (**14**), as it would reduce molecular weight and lipophilicity. In addition, this replacement would decrease the likelihood of inhibitor decomposition via oxidation. Gratifyingly, compounds **13** and **14** had similar potencies, yielding ~50–60% inhibition of SET7 at 40 μM.

We next examined whether additional modifications of the 4-phenyl appended to the indole in **14** improved potency. A carboxylic acid at the *para*-position (**15**) of the phenyl ring greatly suppressed activity, while a trifluoromethyl group (**19**) enhanced efficacy. Other substitutions did not lead to further improvements (Schemes 1C and 2A), making compound **19** the best compound in this series. We then examined whether modification of the indole moiety itself impacted the activity of compounds in this series. To this end, we generated compounds **20**–**24** (Scheme 2B), in which the indole in compound **19** was replaced by pyrrolo-pyrimidine (**20**) and benzimidazole (**21**). As we could not readily access 7-aza-indole and 2-indazole analogs for the precise substitution pattern in compound **19**, we generated analogs (**22**–**24**) in which

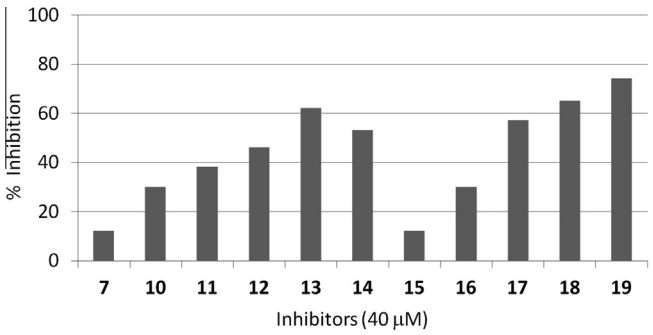

Figure 3. Potency comparison of Indole scaffolds based inhibitors against SET7 in ELISA; inhibition studies were performed with 40 μM inhibitors and DMSO as control ($n \geqslant 2$).