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Targeting lysine specific demethylase 4A (KDM4A) tandem TUDOR domain – A fragment based approach

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ABSTRACT

The tandem TUDOR domains present in the non-catalytic C-terminal half of the KDM4A, 4B and 4C enzymes play important roles in regulating their chromatin localizations and substrate specificities. They achieve this regulatory role by binding to different tri-methylated lysine residues on histone H3 (H3-K4me3, H3-K23me3) and histone H4 (H4-K20me3) depending upon the specific chromatin environment. In this work, we have used a 2D-NMR based fragment screening approach to identify a novel fragment (**1a**), which binds to the KDM4A-TUDOR domain and shows modest competition with H3-K4me3 binding in biochemical as well as *in vitro* cell based assays. A co-crystal structure of KDM4A TUDOR domain in complex with **1a** shows that the fragment binds stereo-specifically to the methyl lysine binding pocket forming a network of strong hydrogen bonds and hydrophobic interactions. We anticipate that the fragment **1a** can be further developed into a novel allosteric inhibitor of the KDM4 family of enzymes through targeting their C-terminal tandem TUDOR domain.

Methylation of lysine residues on histone proteins plays an important epigenetic role in the regulation of gene expression by recruiting different transcription factors to the chromatin.[1–4] Homeostasis of these methylation patterns by different histone lysine methyl transferase and histone lysine demethylase enzymes is of key importance to maintain proper cellular development and to enable cellular differentiation.[3,5] Dysregulation of histone lysine methylations have been linked to aberrant gene expression and malignant transformations.[3,6–8] Removal of the histone lysine methylation marks are catalyzed by two families of enzymes. The first family is comprised of two enzymes namely; lysine specific demethylase 1 (LSD1) and 2 (LSD2). These enzymes belong to the family of flavin dependent amine oxidases.[9–11] The second family is comprised of several enzymes, which are members of the Jumonji C (JmjC) domain containing Fe^{2+} and alpha-ketoglutarate dependent di-oxygenase enzymes.[12,13] These enzymes are also known as JmjC domain containing histone lysine demethylases (JHDMs) and are further classified into seven sub-families based on their sequence similarities and domain architectures.[13]

The KDM4 enzymes, which are expressed as five different isoforms in the human genome (KDM4A, KDM4B, KDM4C, KDM4D and KDM4E), constitute one of the seven sub-families of JHDMs and catalyze demethylation of H3K9 and H3K36 methylation marks.[13–16] Over-expression of KDM4A, 4B and 4C enzymes have been implicated in various cancers making them novel targets for developing anti-cancer drugs.[17–19] All of these enzymes contain the catalytic JmjC domain in their *N*-terminal half, which harbors the conserved Fe^{2+} and alpha-ketoglutarate binding motif. The first three family members (KDM4A, 4B and 4C) also contain a tandem PHD and tandem TUDOR domain in their C-terminal region (see Fig. 1A), which are absent in the KDM4D and KDM4E isoforms. Recent studies have indicated that the tandem TUDOR domains in the KDM4 family of enzymes play a critical role in regulating chromatin localizations and enzymatic function by binding to specific lysine methylation marks on histone proteins (H3-K4me3, H3-K23me3 and H4-K20me3).[6,20–23] Disruption of the TUDOR domain binding to the tri-methylated lysine residues on the histone tail may therefore serve as an allosteric mechanism of antagonizing KDM4 functions under specific chromatin environments.

Various small molecule inhibitors targeting the alpha-ketoglutarate binding site in the catalytic JmjC domain of the KDM4 family of enzymes have been reported with modest potency and

Abbreviations: NMR, nuclear magnetic resonance; ITC, Isothermal Titration Calorimetry; JmjC, Jumonji C; JHDM, JmjC domain containing histone lysine demethylase; CSP, chemical shift perturbation.

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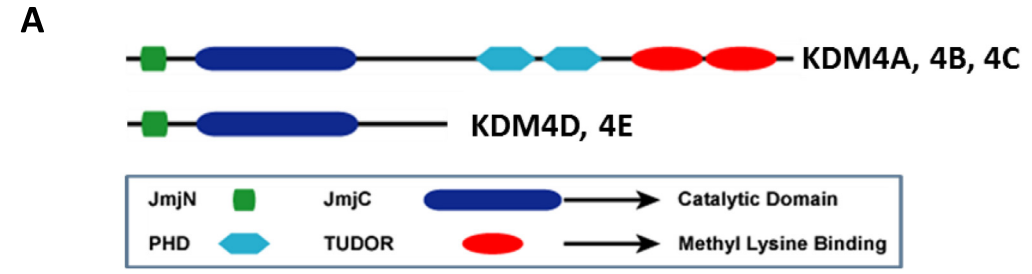

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KDM4A-TUDOR  895  ----ALQSITAGQKVISKHKNGRFYQCEVVRLTTETFYEVNFDDGSFSDNLYPEDIVSQD 950
KDM4B-TUDOR  915  ----LLRAVSLGQVVITKNRNGLYYRCRVIGAASQTCYEVNFDDGSYSDNLYPESITSRD 970
KDM4C-TUDOR  875  ----CEKVISVGQTVITKHRNTRYYSCRVMAVTSQTFYEVMFDDGSFSRDTFPEDIVSRD 930
                       : :: ** **:*::*  :* *.*:  :::* *** *****:* : :**.*.*:*

KDM4A-TUDOR  951  CLQFGPPAEGEVVQVRWTDGQVYGAKFVASHPIQMYQVEFEDGSQLVVKRDDVYTLDEEL 1010
KDM4B-TUDOR  971  CVQLGPPSEGELVELRWTDGNLYKAKFISSVTSHIYQVEFEDGSQLTVKRGDIFTLEEEL 1030
KDM4C-TUDOR  931  CLKLGPPAEGEVVQVKWPDGKLYGAKYFGSNIAHMYQVEFEDGSQIAMKREDIYTLDEEL 990
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Fig. 1. (A) Domain architecture of KDM4A, 4B, 4C, 4D and 4E enzymes. (B) Sequence alignment of the KDM4A, 4B and 4C TUDOR domains.

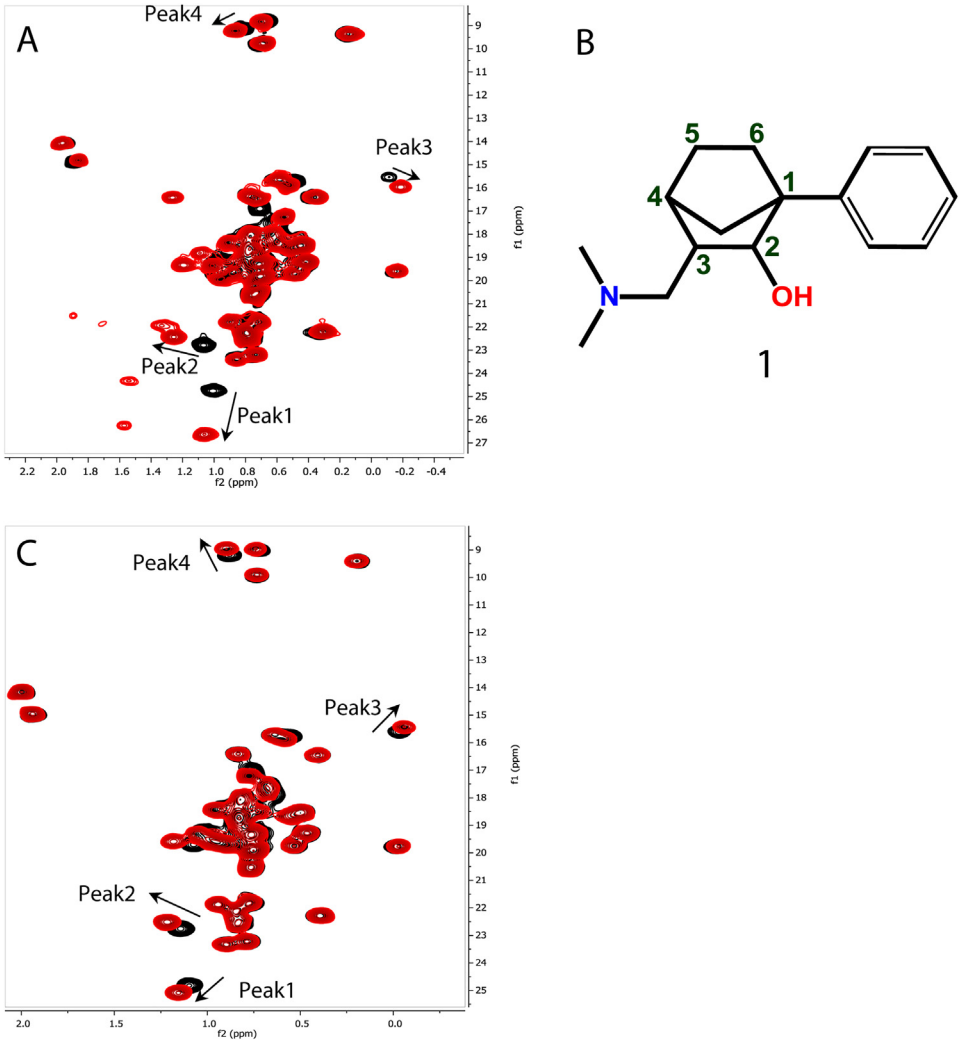

Fig. 2. (A) 13C-HSQC spectra of KDM4A tandem TUDOR domain: Protein only (Black) and with H3(1–10)K4me3 peptide (Red). (B) Fragment **1**: [3-((dimethylamino)methyl)-1-phenylbicyclo[2.2.1]heptan-2-ol]. (C) 13C-HSQC spectra of KDM4A tandem TUDOR: Protein only (Black) and with 1 (Red).