SMYD3 (SET and MYND domain-containing protein 3) is a protein lysine methyltransferase, which was initially described as an H3K4 methyltransferase involved in transcriptional regulation. SMYD3 has recently been reported to methylate and regulate several cancer-relevant non-histone proteins such as mitogen-activated protein kinase kinase kinase 2 (MAP3K2), vascular endothelial growth factor receptor 1 (VEGFR1), RAC-alpha serine/threonine-protein kinase (AKT1), and the human epidermal growth factor receptor 2 (HER2) (Figure 1). Here, we report the discovery of a novel, potent and selective SMYD3 inhibitor BAY-6035.

SMYD3 (SET and MYND domain-containing protein 3) is a protein lysine methyltransferase involved in transcriptional regulation.1 SMYD3 has recently been reported to methylate and regulate several cancer-relevant non-histone proteins such as mitogen-activated protein kinase kinase kinase 2 (MAP3K2,3 RAC-alpha serine/threonine-protein kinase (AKT1,4 HER2, human epidermal growth factor receptor 2; Figure 1. Reported methylation substrates of the SMYD3 methyltransferase.

SMYD3 has recently been reported to methylate and regulate several cancer-relevant non-histone proteins such as mitogen-activated protein kinase kinase kinase 2 (MAP3K2), vascular endothelial growth factor receptor 1 (VEGFR1), RAC-alpha serine/threonine-protein kinase (AKT1), and the human epidermal growth factor receptor 2 (HER2) (Figure 1).

The structure of SMYD3 was determined by using X-ray crystallography, as confirmed by X-ray crystallography (Figure 2). The binding of SMYD3 inhibitors was confirmed by isothermal titration calorimetry (ITC). In addition, surface plasmon resonance (SPR) measurements were done to confirm binding. Kinetic curves were found on a 1:1 binding model. The experiments were performed in the presence of SAM.

The binding geometry of BAY-6035 to its substrate pocket in SMYD3 was determined by using X-ray crystallography, as described previously with slight modifications.

The on-target activity of SMYD3 inhibition was assessed by cellular MAP3K2 methylation assay. HA cells were transfected with HA-tagged MAP3K2 and untreated SMYD3. MAP3K2 methylation was assessed by Western blot using specific in-house generated K260me3 antibody.

Significant improvement of SMYD3 inhibition by X-ray-guided optimization

BAY-6035 demonstrates potent SMYD3 inhibition with an IC50 of 88 nM (Figure 4A) and an equilibrium dissociation constant (Kd) of 100 nM as determined by ITC (Figure 4B) or with SPA (Figure 4C).

BAY-6035 showed highly selective inhibition of SMYD3 with no activity on other methyltransferases (Figure 4D) or kinases (data not shown).

The X-ray structure of BAY-6035 binding is presented in Figure 4E.

ACKNOWLEDGEMENTS

Poster presented at the AACR Annual Meeting 2018, April 14-18, 2018, Chicago, IL, USA

BAY-6035 was identified as a highly potent and selective SMYD3 inhibitor by the optimization of a primary benzodiazepine hit cluster. The binding geometry of BAY-6035 to its substrate pocket in SMYD3 was unambiguously characterized by X-ray crystallography.

BAY-6035 specifically inhibited cellular methylation of MAP3K2 by SMYD3. In conclusion, BAY-6035 is a novel, selective and potent SMYD3 inhibitor probe and will foster a better understanding of the biological role of SMYD3 in cancer (Figure 6).


REFERENCES