Identification of a Novel Potent Selective SMYD3 Inhibitor with Oral Bioavailability


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Background

SET and MYND Domain containing 3 (SMYD3) is a lysine methyltransferase (KMT) expressed at high levels in a number of different cancer histologies and is associated with a poor clinical prognosis.1, 2 While no single mechanism has emerged to explain this correlation, a number of studies have implicated SMYD3 in the regulation of gene transcription and signal transduction pathways critical for cell survival in breast, liver, prostate, pancreatic and lung cancer models.3-5, 11 In addition, considerable evidence has been reported in the literature showing that genetic knockdown of SMYD3 leads to a decrease in proliferation of a variety of cancer cell lines.6-8, 14 Two studies, employing RNAi-based technologies have shown that ablation of SMYD3 in hepatocellular carcinoma cell lines greatly reduces cell viability and that its pro-oncogenic role is dependent on its catalytic activity.9, 10 Moreover, SMYD3 has also been shown to be a critical mediator of transformation induced by a KRAS gain-of-function mutation in both pancreatic and lung adenocarcinoma mouse models; these models were likewise dependent on the catalytic activity of SMYD3.

SMYD3 role in cancer cell proliferation, its effect on known oncogenic signal transduction pathways and the association of SMYD3 mRNA expression with aggressive transformed phenotypes makes SMYD3 an attractive target for therapeutic intervention.

Results

Crystal structure of EPZ030456 in complex with SMYD3 and SAM

Starting SMYD3 hit, compound 1, and initial SAR

Sulfonamide SAR

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>SMYD3 inhibition IC50 (nM) a</th>
<th>IC50 (nM) b</th>
</tr>
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<td>H</td>
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<td>84 +/− 0</td>
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<td>H</td>
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</table>

EPZ030456 and EPZ031686 were further characterized:

EPZ030456 and EPZ031686 show tight binding kinetics to SMYD3 by SPR

EPZ030456 and EPZ031686 show endogenous SMYD3 target engagement in cellular thermal shift assay

HCC proliferation activity

SMYD3 inhibitors did not show activity against 43 hepatocellular carcinoma cell lines.

EPZ030456 and EPZ031686 are selective for SMYD3

EPZ030456 and EPZ031686 show <30% inhibition against 16 HMT's (DOT1L, EHMT1, EHMT2, EZH2, NSD1, PRDM9, PRMT3, PRMT7, PRMT8, SETD7, SETDB1, SUZ39H1, WHSC1, WHSC1L1) at a 10 μM screening concentration. Both compounds have IC50 > 50 μM against highly homologous SMYD2.

Blood pharmacokinetic parameters for EPZ031686 following i.p. administration to male CD-1 mice. Expressed as mean ± SD, n=3.

Parameter EPZ030456 EPZ031686

Bioavailability (F) of 69% following p.o. dose at 50 mg/kg led to EPZ031686 unbound blood concentration remaining above the SMYD3 IC50 value for more than 12 h, thus, amenable for murine pharmacology models.

Conclusions

• EPZ030456 and EPZ031686 are potent selective small molecule inhibitors of SMYD3 with cellular IC50 < 50 nM.

• Crystallography shows EPZ030456 binds in the SMYD3 substrate site with the oxindole head-group occupying the Lys channel.

• EPZ031686 shows good bioavailability following oral dosing in mice making it a suitable tool compound for in vivo target validation studies.

References


2. Liu, Y.; Liu, H.; Luo, X.; Deng, J.; Pan, Y.; Liang, H., Overexpression of SMYD3 and matrix metalloproteinase-9 are associated with poor prognosis of patients with gastric cancer. (2), 36

3. Liu, Y.; Deng, J.; Luo, X.; Pan, Y.; Zhang, L.; Zhang, R.; Liang, H., Overexpression of SMYD3 was associated with increased STAT3 activation in gastric cancer. (3), 97


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9. Heffner, J.; Jeronimo, C., SMYD3's role in cancer cell line proliferation, its

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