#C85

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.[1-10] 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.[4, 7-9]

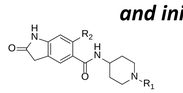
In addition, considerable evidence has been reported in the literature showing that genetic knockdown of SMYD3 leads to a decrease in proliferation of a variety of cancer cell lines.[4, 7-9, 11] Two studies, employing RNAi-based technologies have shown that ablation of SMYD3 in hepatocellular carcinoma cell lines greatly reduces cell viability and that its pro-oncogenic role is dependent on its catalytic activity.[7, 9] 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.[11]

SMYD3's role in cancer cell line 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

- Compound **1** was identified as a micromolar inhibitor of SMYD3 through screening of Epizyme's proprietary histone methyltransferase-biased library.
- A 1.5 Å resolution crystal structure of **1** was solved in a ternary complex with SMYD3 and SAM and shows the oxindole head-group occupies the lysine channel of SMYD3.
- The piperidine tail of **1** is positioned in the large solvent filled peptide binding site and engages in a water mediated interaction with Glu192.

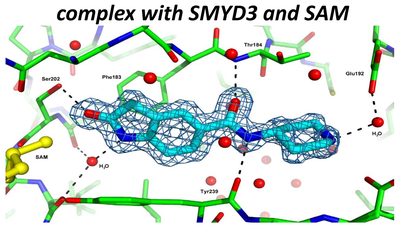
Starting SMYD3 hit, compound 1, and initial SAR



#	R1	R2	SMYD3 biochemical IC_{50} (nM) [a]	SMYD3 ICW IC_{50} (nM) [a]
1	H	H	17000	>10000
2	$SO_2(CH_2)_3NH_2$	H	160	>10000
3	$SO_2(CH_2)_3NH_2$	Cl	80	6800
4		H	70	>10000

[a] IC_{50}'s were determined from at least two independent experiments. [b] Trimethyl MEKK2 In Cell Western Assay.

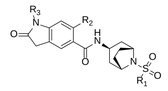
Crystal structure of Compound 1 in complex with SMYD3 and SAM



Structure of **1** (cyan) in complex with SMYD3 (green) and SAM (yellow) (PDB ID 5CCL). Electron density (2Fo-Fc, 1σ) for the compound is shown. Hydrogen bonds are indicated as dashed lines.

Results

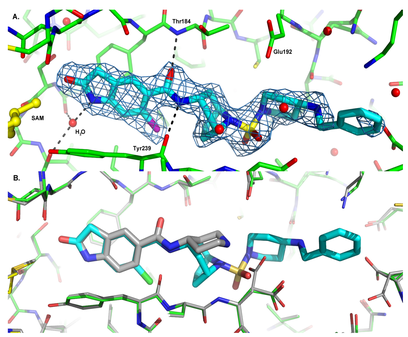
- Initial analog design focused on introduction of a basic center to pick up a direct interaction with either Glu192 or Asp241 which are both within 3.5 Å of the piperidine nitrogen.



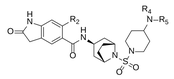
Sulfonamide SAR

#	R1	R2	R3	SMYD3 biochemical IC_{50} (nM) [a]	SMYD3 ICW IC_{50} (nM) [b]
5		H	H	4	3400
6		Cl	H	4	3200
7		H	H	1	480
8		H	Me	26	3900
EPZ031686		Cl	H	3	36
9		Cl	H	2	760
10		Cl	H	5	730
11		Cl	H	4	1300
12		H	H	4	2200

Crystal structure of EPZ030456 in complex with SMYD3 and SAM



A. Structure of **EPZ030456** (cyan) with SMYD3 (green) and SAM (yellow) (PDB ID 5CCM). Electron density (2Fo-Fc, 1σ) for the compound is shown. Hydrogen bonds are indicated as dashed lines. B. The headgroup of **EPZ030456** (cyan) superimposes well with the oxindole of compound 1 (grey), while the saturated ring linkers take divergent paths in the active site.

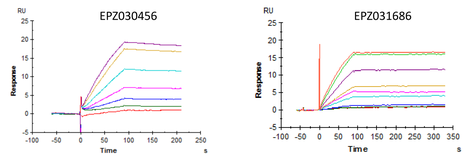


Sulfamide SAR

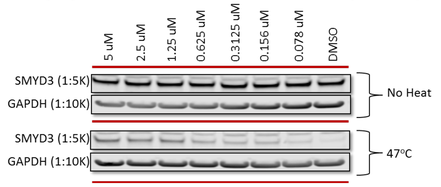
#	R2	R4	R5	SMYD3 biochemical IC_{50} (nM) [a]	SMYD3 ICW IC_{50} (nM) [b]
13	Cl	H	H	1	1100
14	Cl	H	Me	2	420
15	Cl	Me	Me	3	300
EPZ030456	Cl	H	Bn	4	48
16	F	H	H	5	530
17	Me	H	H	16	410
18	H	H	H	1	850

- Cell potent compounds EPZ030456 and EPZ031686 were further characterized:

EPZ030456 and EPZ031686 show tight binding kinetics to SMYD3 by SPR



EPZ030456 shows 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

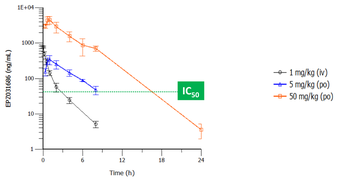
- EPZ030456 and EPZ031686 show <30% inhibition against 16 HMT's (DOT1L, EHMT1, EHMT2, EZH1, EZH2, NSD1, PRDM9, PRMT3, PRMT6, PRMT7, PRMT8, SETD7, SETDB1, SUV39H1, WHSC1, WHSC1L1) at a 10 µM screening concentration. Both compounds have IC_{50} > 50 µM against highly homologous SMYD2.

EPZ030456 and EPZ031686 in vitro *ADME profile:*

Parameter	EPZ030456	EPZ031686
MLM CL (mL/min/kg)	34	24
Caco-2 A to B (x10^{-6} cm/s)	0.34 ± 0.22	0.64 ± 0.20
Caco-2 Efflux ratio	104	41
Mouse Fu	0.32 ± 0.04	0.53 ± 0.12

- **EPZ031686** has a more favorable *in vitro* ADME profile than **EPZ030456** so was taken on to PK studies.

PK profile of EPZ031686:



Mean total blood concentration-time profiles of EPZ031686 after an IV dose of 1 mg/kg and SC doses of 5 and 50 mg/kg in male C1-1 mice, n=3, mean ± SD.

Parameter	1 mg/kg IV	5 mg/kg p.o.	50 mg/kg p.o.
CL (mL/min/kg)	27 ± 3.9	-	-
CL_r (mL/min/kg)	5.3 ± 1.6	-	-
V_{ss} (L/kg)	2.3 ± 0.29	-	-
$t_{½}$ (h)	1.7 ± 0.13	2.7 ± 0.98	2.2 ± 0.09
t_{max} (h)	-	0.89 ± 0.19	1.3 ± 0.58
C_{max} (µg/mL)	-	0.35 ± 0.09	4.7 ± 0.08
$AUC_{0\text{-}last}$ (h*µg/mL)	0.60 ± 0.09	1.28 ± 0.24	21.2 ± 0.25
F (%)	-	48 ± 5.4	69 ± 8.2

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

- Bioavailability (F) of 69% following p.o. dose at 50 mg/kg led to **EPZ031686** unbound blood concentration remaining above the SMYD3 ICW IC_{50} 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 IC_{50} < 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.

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Disclosures: Epizyme authors: Epizyme employment, Epizyme equity ownership