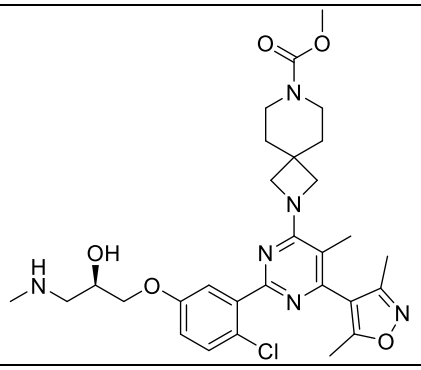
Product data sheet

MedKoo Cat#: 562180 Name: EZM2302 CAS#: 1628830-21-6 Chemical Formula: $C_{29}H_{37}ClN_6O_5$ Exact Mass: 584.2514 Molecular Weight: 585.1	
Product supplied as:	Powder
Purity (by HPLC):	≥ 98%
Shipping conditions	Ambient temperature
Storage conditions:	Powder: -20°C 3 years; 4°C 2 years.
	In solvent: -80°C 3 months; -20°C 2 weeks.

1. Product description:
EZM2302, also known as GSK3359088, is a potent, selective, and orally available arginine methyltransferase CARM1 inhibitor.

2. CoA, QC data, SDS, and handling instruction
SDS and handling instruction, CoA with copies of QC data (NMR, HPLC and MS analytical spectra) can be downloaded from the product web page under "QC And Documents" section. Note: copies of analytical spectra may not be available if the product is being supplied by MedKoo partners. Whether the product was made by MedKoo or provided by its partners, the quality is 100% guaranteed.

3. Solubility data

Solvent	Max Conc. mg/mL	Max Conc. mM
DMSO	91.0	155.53

4. Stock solution preparation table:

Concentration / Solvent Volume / Mass	1 mg	5 mg	10 mg
1 mM	1.71 mL	8.55 mL	17.09 mL
5 mM	0.34 mL	1.71 mL	3.42 mL
10 mM	0.17 mL	0.85 mL	1.71 mL
50 mM	0.03 mL	0.17 mL	0.34 mL

5. Molarity Calculator, Reconstitution Calculator, Dilution Calculator
Please refer the product web page under section of "Calculator"

6. Recommended literature which reported protocols for in vitro and in vivo study
In vitro study

1. Drew AE, Moradei O, Jacques SL, Rioux N, Boriack-Sjodin AP, Allain C, Scott MP, Jin L, Raimondi A, Handler JL, Ott HM, Kruger RG, McCabe MT, Sneeringer C, Riera T, Shapiro G, Waters NJ, Mitchell LH, Duncan KW, Moyer MP, Copeland RA, Smith J, Chesworth R, Ribich SA. Identification of a CARM1 Inhibitor with Potent In Vitro and In Vivo Activity in Preclinical Models of Multiple Myeloma. Sci Rep. 2017 Dec 21;7(1):17993. doi: 10.1038/s41598-017-18446-z. PMID: 29269946; PMCID: PMC5740082.

In vivo study

1. Drew AE, Moradei O, Jacques SL, Rioux N, Boriack-Sjodin AP, Allain C, Scott MP, Jin L, Raimondi A, Handler JL, Ott HM, Kruger RG, McCabe MT, Sneeringer C, Riera T, Shapiro G, Waters NJ, Mitchell LH, Duncan KW, Moyer MP, Copeland RA, Smith J, Chesworth R, Ribich SA. Identification of a CARM1 Inhibitor with Potent In Vitro and In Vivo Activity in Preclinical Models of Multiple Myeloma. Sci Rep. 2017 Dec 21;7(1):17993. doi: 10.1038/s41598-017-18446-z. PMID: 29269946; PMCID: PMC5740082.

7. Bioactivity
Biological target:
EZM 2302 is an inhibitor of coactivator-associated arginine methyltransferase 1 (CARM1) with an IC50 of 6 nM.

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In vitro activity

To assess the cellular activity of EZM2302 in vitro, changes in cellular methylation levels upon treatment with CARM1 inhibitor were quantified. The effect of EZM2302 treatment on cellular methylation was tested by immunoblot in the multiple myeloma (MM) cell line RPMI-8226 (Fig. 3a, Supplementary Fig. S2). Methylation changes were measured at the well-characterized CARM1 substrates PABP1 and SmB. Ninety-six-hour EZM2302 treatment led to a concentration-dependent decrease in methylation of PABP1 ($IC50 = 0.038 \pm 0.015$ μM, $N = 3$) and SmB (increased levels of SmBme0, $EC50 = 0.018 \pm 0.007$ μM, $N = 3$), as well as in multiple aDMA bands ($IC50 = 0.009$ μM). Similar results were also observed in the NCI-H929 (Fig. 3b and Supplementary Fig. S2) and U266B1 MM cell lines (Supplementary Fig. S5,S6). The effects of CARM1 inhibition on cellular histone methylation at the putative CARM1 substrates H3R17 and H3R26 were also evaluated by performing western blot analysis on whole cell lysates. In summary, a novel series has been identified as CARM1 inhibitors. Multi-parametric chemical optimization resulted in compound EZM2302, which exhibited nanomolar biochemical activity against CARM1 that was well correlated with both cellular target engagement and in vitro anti-proliferative effect, and shows exquisite selectivity against other HMTs with no off-target activities.

Reference: Sci Rep. 2017; 7: 17993. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5740082/

In vivo activity

A dose range-finding (DRF) study conducted for 7 days with twice daily (BID) oral dosing of EZM2302 in CB-17 SCID mice at 37.5–300 mg/kg showed that all doses were well-tolerated with minimal body weight loss (Supplementary Fig. S10). To understand the kinetics of in vivo target inhibition, levels of PABP1, SmB, and aDMA methylation were assessed in tumor tissue after 2, 4, and 8 days of EZM2302 treatment at 150 and 300 mg/kg BID, based on the tolerability of these doses in the DRF. Inhibition of PAPB1me2a and aDMA and induction of SmBme0 were robustly observed within two days of dosing, with maximal changes in methylation observed at day 4 in both dose groups (Fig. 4e and Supplementary Fig. S11). EZM2302 showed dose-dependent exposure and tumor growth inhibition (TGI) after 21 days in the RPMI-8226 xenograft model (Fig. 5a). Tumors in all EZM2302 dose groups measured on day 21 showed significant decreases in tumor growth compared to vehicle (2-way ANOVA compared to Vehicle, Dunnett's post-test). Tumor growth inhibition ranged from 45% in the 37.5 mg/kg dose group to 63% in the 300 mg/kg dose group. RPMI-8226 xenograft tumors collected on day 21 showed a dose-dependent decrease in methylation at all tested CARM1 substrates (Fig. 5b,c and Supplementary Fig. S12). A statistically significant increase in unmethylated form of SmB (SmBme0) was detected at all dose groups, from an 8-fold increase at 37.5 mg/kg to a 14-fold increase at 150 mg/kg. aDMA levels were likewise significantly decreased at all dose groups, a maximal inhibition of 65% was observed at the 75 mg/kg dose group. In summary, EZM2302 is a tool compound that can be used to further explore the biological role of CARM1 and understand the role of this enzyme in multiple myeloma and other oncology indications.

Reference: Sci Rep. 2017; 7: 17993. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5740082/