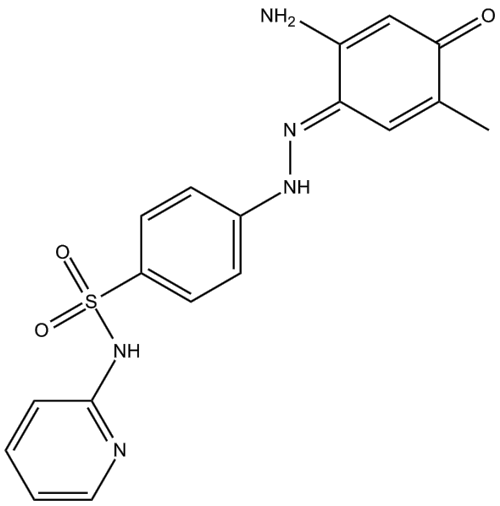
Product Data Sheet

Product Name: MS436
Cat. No.: GC13148

Chemical Properties

Cas. No.	1395084-25-9		
Chemical Name	4-[(2Z)-2-(2-amino-5-methyl-4-oxocyclohexa-2,5-dien-1-ylidene)hydrazinyl]-N-pyridin-2-ylbenzenesulfonamide		
SMILES	CC1=CC(=NNC2=CC=C(C=C2)S(=O)(=O)NC3=CC=CC=N3)C(=CC1=O)N		
Formula	C18H17N5O3S	M.Wt	383.42
Solubility	≥ 19.15mg/mL in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



Protocol

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Kinase experiment: Binding affinity of the newly synthesized diazobenzene compounds (e.g., MS436) for various bromodoamins is assessed in a fluorescence anisotropy competition assay using a fluorescein isothiocyanate (FITC)-labeled MS417 as an assay probe. Competition experiments are performed with a BrD protein (0.25-1 μM) and the fluorescent probe (80 nM), and increasing concentration of unlabeled competing ligand in a PBS buffer (pH 7.4) in total volume of 80 μL Measurements are obtained after a 1 hour incubation of the fluorescent ligand and the protein at 25°C with Safire 2 microplate reader. In a competition-binding assay, fluorescent ligand concentration is ≤2Kd, and protein concentration is set at which 50-80% of fluorescent ligand is bound. Dissociation constant of a competing ligand is calculated with the correction to Cheng-Prussoff equation introduced by Nicolovska-Coleska and colleagues. Assuming one-site competitive binding model, the equation used to calculate Ki's from IC50 values[1].

Cell experiment: Murine macrophage RAW264.7 cells are plated at a density of 1×104 cells per well in a 96-well plate and incubated at 37°C for 18 h. The cells are then treated with the diazobenzene bromodomain inhibitors (e.g., MS436) up to 100 μM for 24 hours. At the end of the 24 hr incubation, 10 μL of the MTT solution (4 mg/mL) is added to each well and incubated at 37°C for 4 h. The supernatants are then removed and the cells are solubilized in 100 μL of 100% DMSO. The diazobenzene compounds are first dissolved in DMSO then diluted with culture medium to concentrations that ranged from 0.28 to 50000 nM. The final concentration of DMSO is adjusted to 0.05% (v/v). The extent of the reduction is measured by the absorbance at 570/630 nm using EnVison 2104 Multilabel Reader[1].

Caution: Product has not been fully validated for medical applications. For research use only.
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References:

[1]. Zhang G, et al. Structure-Guided Design of Potent Diazobenzene Inhibitors for the BET Bromodomains. J Med Chem. 2013 Nov 27;56(22):9251-64.

Background

MS436 is a potent and selective small-molecule inhibitor of BRD4 with Ki values of ＜0.085μM and 0.34μM, respectively for BrD1 and BrD2 [1].

BRD4 plays a role in gene transcription and is a drug target for cancer and inflammation. It has two bromodomains. MS436 is a diazobenzene compound, it is designed from the SAR studies to have higher selectivity. In vitro fluorescent anisotropy assay shows MS436 has about 10-fold higher affinity of BrD1 over BrD2. MS436 binds to BRD4 through a set of water-mediated interaction and this is the molecular basis for the binding affinity. MS436 also has activity to CBP BrD. In RAW264.7 cells, MS436 can block NF-κB-directed NO production and block the expression of proinflammatory cytokine interleukin (IL)-6 induced by LPS [1].

References:
[1] Zhang G, Plotnikov AN, Rusinova E, Shen T, Morohashi K, Joshua J, Zeng L, Mujtaba S, Ohlmeyer M, Zhou MM. Structure-guided design of potent diazobenzene inhibitors for the BET bromodomains. J Med Chem. 2013 Nov 27;56(22):9251-64.

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