Product Data Sheet

Product Name: BB-Cl-Amidine
Cat. No.: GC31755

Chemical Properties

Cas. No.	1802637-39-3		
SMILES	O=C(C1=CC=C(C2=CC=CC=C2)C=C1)N[C@H](C3=NC4=CC=CC=C4N3)CCCNC(CCl)=N		
Formula	$C_{26}H_{26}ClN_5O$	M.Wt	459.97
Solubility	DMSO : 125 mg/mL (271.76 mM)	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ 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	BB-Cl-Amidine		

Protocol

Cell experiment [1]:

Cell lines	MCF10DCIS cells
Preparation Method	MCF10DCIS cells were grown in soft agar at different concentrations of BB-CL-AMIDINE (0 μM (DMSO), or 1 μM BB-CL-AMIDINE). After 2.5 weeks, individual colonies larger than 70 μm were counted.
Reaction Conditions	0 μM or 1 μM; 2.5 weeks

Caution: Product has not been fully validated for medical applications. For research use only.
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Applications	There was an average of 3,536 colonies in the DMSO control whereas only 1,967 colonies were seen in the BB-CL-AMIDINE treated group after 2.5 weeks of soft agar culture. This represents a 44% decrease in the average colony formation in the presence of 1 μM BB-CL-AMIDINE, indicating a significant tumorigenic inhibition of breast cancer cells (MCF10DCIS cells) by the PADI inhibitor.

Animal experiment [2]:

Animal models	Eight-week-old female NOD mice
Dosage form	1 μg/g; s.c.
Preparation method	Eight-week-old female NOD mice were used. Treatments involved subcutaneous injections with BB-Cl-amidine (1 μg/g body weight) or vehicle (25% DMSO in PBS) six times per week until 25 weeks of age for diabetes incidence or until 13 weeks of age for mechanistic studies
Applications	BB-Cl-amidine treatment fully prevented diabetes development, with all mice free of diabetes until 25 weeks of age, against 44% diabetes free in DMSO-treated mice.

References:

[1] Horibata S, et al. Utilization of the Soft Agar Colony Formation Assay to Identify Inhibitors of Tumorigenicity in Breast Cancer Cells. J Vis Exp. 2015 May 20;(99):e52727.

[2] Sodré FMC, et al. Peptidylarginine Deiminase Inhibition Prevents Diabetes Development in NOD Mice. Diabetes. 2021 Feb;70(2):516-528.

Background

BB-Cl-Amidine, a peptidylarginine deminase (PAD) inhibitor, is frequently used to study PAD function.[1]

In vitro experiment it shown that after 48 h of treatment with 0 to 20 μM BB-Cl-Amidine caused a dose-dependent decrease in cell viability in canine and feline mammary tumor cells.[1] In vitro, Cl-amidine and BB-Cl-Amidine show similar potencies and selectivities in U2OS cells, the cellular potency of BB-Cl-Amidine is increased by more than 20-fold, with EC50 values of 8.8±0.6 μM in U2OS osteosarcoma cells.[5]

In vivo efficacy test it exhibited that treatment with 1 μg/ml BB-Cl-Amidine intraperitoneally for two weeks in xenograft mice, BB-Cl-Amidine-treated tumors became crusty and the surrounding skin showed hair loss. There was an or a slight increase in apoptotic cells in the BB-Cl-Amidine-treated canine or feline xenograft tumors.[1] In vitro, at concentrations around 15-20 μM and 4 μM, respectively, BB-Cl-Amidine inhibited both PAD isoforms in a dose-dependent manner with 90% inhibition of PAD2 and

PAD4.[2]

In vivo, arthritic mice were treated with 10 mg/kg BB-Cl-Amidine, there was a reduction in inflammation and joint destruction.[3] In vivo, treatment with 1 mg/kg BB-Cl-Amidine intraperitoneally and Ac-YVAD-cmk (a pyroptosis inhibitor) attenuated NET levels in BALF and neutrophil infiltration in alveoli. [4] In vivo, treatment with 1 mg/kg BB-Cl-Amidine subcutaneously obviously reduced splenomegaly in MRL/lpr mice and improved endothelium-dependent vasorelaxation.[5]

References:
[1] Ledet MM, et al. BB-Cl-Amidine as a novel therapeutic for canine and feline mammary cancer via activation of the endoplasmic reticulum stress pathway. BMC Cancer. 2018 Apr 12;18(1):412.
[2] Martín Monreal MT, et al. Applicability of Small-Molecule Inhibitors in the Study of Peptidyl Arginine Deiminase 2 (PAD2) and PAD4. Front Immunol. 2021 Oct 19;12:716250.
[3] Kawalkowska J, et al. Abrogation of collagen-induced arthritis by a peptidyl arginine deiminase inhibitor is associated with modulation of T cell-mediated immune responses. Sci Rep. 2016 May 23;6:26430.
[4] Li H, Li Y, et al. Neutrophil Extracellular Traps Augmented Alveolar Macrophage Pyroptosis via AIM2 Inflammasome Activation in LPS-Induced ALI/ARDS. J Inflamm Res. 2021 Sep 21;14:4839-4858.
[5] Knight JS, et al. Peptidylarginine deiminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. Ann Rheum Dis. 2015 Dec;74(12):2199-206.