

Product Data Sheet

Product Name: Ac-ANW-AMC

Cat. No.: GC42685

Chemical Properties

Cas. No. 2357123-49-8

Chemical Name (S)-N1-((S)-3-(1H-indol-3-yl)-1-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-1-oxopropan-2-yl)-2-((S)-2-acetamidopropanamido)succinamide

SMILES O=C1C=C(C)C2=C(C=C(NC([C@H](CC3=CNC4=C3C=CC=C4)NC([C@H](CC(N)=O)NC([C@H](C)NC(C)=O)=O)=O)=O)C=C2)O1

Formula C30H32N6O7

M.Wt 588.6

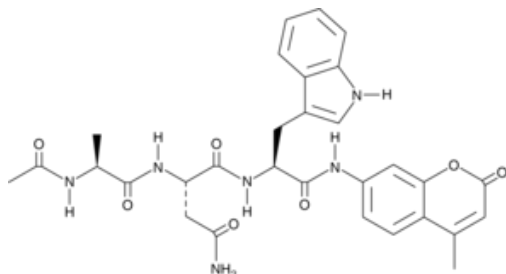
Solubility 10mM in DMSO

Storage Store at -20°C, protect from light

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

This plan only provides a guide, please modify it to meet your specific needs.

1. Solution preparation

(1) Working solution: Dissolve Ac-ANW-AMC in DMSO and dilute to the required working concentration with assay buffer, e.g. 30-100µM^[1, 2].

Note: Please adjust the optimal working concentration according to the actual situation or refer to the literature to set the gradient concentration by yourself. The working solution must be prepared and used immediately.

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

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2. Measure the catalytic activity of $\beta 5i$ by monitoring the hydrolysis rate of Ac-ANW-AMC over time^[1] (from the literature, for reference only)

(1) Prepare protein lysate using Passive Lysis Buffer and dilute in 20S proteasome assay buffer (20mM Tris-HCl, 0.5mM EDTA, 0.035% SDS, pH 8.0).

(2) Add 100 μ M Ac-ANW-AMC to initiate the catalytic reaction of $\beta 5i$.

(3) The fluorescence signal was measured using a microplate reader at an excitation wavelength of 360nm and an emission wavelength of 460nm, with readings taken once per minute for 1h.

3. Determination of proteasomal LMP7 activity using Ac-ANW-AMC^[2] (from the literature, for reference only)

(1) The reaction mixture contained 20mM Tris-HCl (pH 7.5), 30 μ M Ac-ANW-AMC, 1mM dithiothreitol (DTT), 5mM MgCl₂, and 1mM ATP.

(2) To calculate the contribution of non-proteasomal proteolytic activity, 7 μ M MG 132 (a proteasome inhibitor) was used. After adding 0.5, 1.0, 1.5, and 2.0 μ L of the clarified homogenate to a total volume of 100 μ L, the reaction was incubated at 37°C for 20min and terminated with 1% SDS.

(3) The product was detected using a fluorometer with an excitation wavelength of 380nm and an emission wavelength of 440nm. The difference between the total activity and the residual activity in the presence of MG 132 was determined as the proteasome activity.

References:

[1] Lee M J, Miller Z, Park J E, et al. H727 cells are inherently resistant to the proteasome inhibitor carfilzomib, yet require proteasome activity for cell survival and growth[J]. Scientific reports, 2019, 9(1): 4089.

[2] Erokhov P A, Kulikov A M, Karpova Y D, et al. Proteasomes in patient rectal cancer and different intestine locations: where does proteasome pool change?[J]. Cancers, 2021, 13(5): 1108.

Background

Ac-ANW-AMC is a fluorescent substrate for the $\beta 5i$ (LMP7/PSMB8) subunit of the 20S immunoproteasome, with a maximum excitation wavelength of λ_{Ex} =345nm and a maximum emission wavelength of λ_{Em} =445nm^[1, 2]. When a specific enzyme acts on Ac-ANW-AMC, it releases the fluorophore 7-amido-4-methylcoumarin (AMC). The activity of the enzyme can be quantitatively analyzed by measuring the fluorescence intensity of AMC^[3]. The fluorescence signal of Ac-ANW-AMC can be effectively eliminated by the i-proteasome inhibitor ONX-0914^[4].

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- [1] Winter M B, La Greca F, Arastu-Kapur S, et al. Immunoproteasome functions explained by divergence in cleavage specificity and regulation[J]. Elife, 2017, 6: e27364.
- [2] Erokhov P A, Kulikov A M, Karpova Y D, et al. Proteasomes in patient rectal cancer and different intestine locations: where does proteasome pool change?[J]. Cancers, 2021, 13(5): 1108.
- [3] Mustafa M S, El-Abadelah M M, Mubarak M S, et al. Synthesis and fluorogenic properties of some 1-(coumarin-7-yl)-4, 5-dihydro-1, 2, 4-triazin-6 (1H)-ones[J]. International Journal of Chemistry, 2011, 3(4): 89.
- [4] Kim S, Park S H, Choi W H, et al. Evaluation of Immunoproteasome-Specific Proteolytic Activity Using Fluorogenic Peptide Substrates[J]. Immune Network, 2022, 22(3).

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