Product Information

Leupeptin

Trifluoroacetate salt, microbial, ≥ 90% (HPLC)

L2023

Product Description

CAS Number: 147385-61-3

Synonyms: *N*-Acetyl-L-leucyl-L-leucyl-L-argininal trifluoroacetate salt, Acetyl-Leu-Leu-Arg-al

Molecular Weight: 426.55 (free base basis)

Molecular Formula: $C_{20}H_{38}N_6O_4 \bullet x\ C_2HF_3O_2$

Leupeptin is a reversible competitive inhibitor of cysteine proteases and serine proteases.[1] Leupeptin acts by covalent binding to, respectively:[2]

- Catalytic cysteines of cysteine proteases
- Catalytic series of serine proteases

Leupeptin was first isolated from microbial sources as a mixture of two very similar forms:[3]

- Acetyl-Leu-Leu-Arg-al
- Propionyl-Leu-Leu-Arg-al

While the propionyl leupeptin is active as an inhibitor, the acetyl form is more commonly used.

Leupeptin has been reported to inhibit calpain,[4] cathepsin B,[5] cathepsins H and L,[6] and trypsin.[7] A typical working concentration range is 10-100 µM. The activity of leupeptins and related analogs has been studied.[9] Table 1 lists inhibitory activities of leupeptin against various enzymes.[10]

HPLC analysis of leupeptin gives multiple peaks because of the formation of tautomeric isomers in solution.[11] The primary mechanism of inactivation of leupeptin is via racemization of the L-arginal moiety, as leupeptin with a D-arginal group is totally inactive.[10] If the aldehyde is oxidized but retains its L-configuration, the resulting carboxylate compound does have some inhibitory activity.[12]

Several theses[13] and dissertations[14-16] have cited use of product L2023 in their protocols.

Table 1. Concentrations for 50% inhibition (IC_{50}, reported as µg/mL leupeptin)[10]

Enzyme	Substrate	IC_{50} (µg/mL)
Aspergillopepsin II (Proctase A)	Casein	> 250
Aspergillopepsin I (Proctase B)	Casein	> 250
Cathepsin A	Carbobenzoxy-L·glutamyl-L·tyrosine (Cb-Glut-Tyr)	1680
Cathepsin B	*N*α-benzoyl-L-arginine amide HCl	0.44
Cathepsin D	Hemoglobin	109
α-Chymotrypsin	Casein	> 500
β-, γ-, and δ-Chymotrypsin	Casein	> 500
Kallikrein	BAEE (*N*α-benzoyl-L-arginine ethyl ester HCI)	75
Papain	Casein	0.5
Pepsin	Casein	> 500
	Hemoglobin	> 500
Plasmin	Fibrinogen	8
Thrombin	*N*α-(*p*-toluene-sulfonyl)-L-arginine methyl ester HCl	10000
Thrombokinase	Plasma	15
Trypsin	Casein	2

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the lyophilized product at –20 °C.

Solubility

This product is tested for solubility in water at 50 mg/mL.

A 10 mM aqueous solution of leupeptin has been reported to be stable for a week at 4 °C, and for a month at –20 °C.[8] At working concentrations (10-100 µM), a solution is stable for only a few hours.[8] The stock solution should be stored on ice for intermittent use over several hours.

Usage

Because of its aldehyde group, leupeptin may act as a reducing agent, and thus may interfere in protein determination assays, such as the Lowry assay and, to a lesser extent, the Bradford assay.

References

1. Umezawa, H., *Methods Enzymol.*, **45**, 678-695 (1976).
2. Kędzior, M. *et al.*, *Med. Microbiol. Immunol.*, **205(4)**, 275-279 (2016).
3. Aoyagi, T. *et al.*, *J. Antibiot. (Tokyo)*, **22(6)**, 283-286 (1969).
4. Zimmerman, U-J.P. and Schlaepfer, W.W., *Biochemistry*, **21(17)**, 3977-3983 (1982).
5. Knight, C.G., *Biochem. J.*, **189(3)**, 447-453 (1980).
6. Zollner, H., *Handbook of Enzyme Inhibitors*, 2nd ed., Part B. VCH Press, pp. 821-822 (1993).
7. Kuramochi, H. *et al.*, *J. Biochem.*, **86(5)**, 1403-1410 (1979).
8. Beynon, R., and Bond, J.S., *Proteolytic Enzymes: A Practical Approach* (2nd ed.). Oxford University Press (Oxford, UK), p. 323 (2001).
9. Maeda, K. *et al.*, *J. Antibiotics*, **24(6)**, 402-404 (1971).
10. Umezawa, H., *Pure Appl. Chem.*, **33(1)**, 129-144 (1973).
11. Saino, T. *et al.*, *Chem. Pharm. Bull.*, **30(7)**, 2319-2325 (1982).
12. Billinger, E. *et al.*, *FEBS Open Bio.*, **10(12)**, 2605-2615 (2020).
13. Griffin, Carly, "Evaluation of the Selective Anti-Cancer Activity of Natural and Synthetic Alkaloids". University of Windsor, M.Sc. thesis, p. 179 (2011).
14. McGrowder, Eva, "The Role of the Metastasis Suppressor Tetraspanin CD82/KAI1 in Trastuzumab-Mediated Cellular Responses". University of Birmingham, Ph.D. dissertation, p. 240 (2012).
15. Busch, Johanna Irene, "Increased Expression of Frontotemporal Dementia Risk Factor Tmem106b Alters Lysosomal and Autophagosomal Pathways". University of Pennsylvania, Ph.D. dissertation, p. 105 (2016).
16. Hundley, Frances, "A comprehensive phenotypic CRISPR-Cas9 screen of the ubiquitin pathway uncovers roles of ubiquitin ligases in mitosis". University of California San Francisco, Ph.D. dissertation, p. 52 (2020).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

L2023pis Rev 07/22 CKV,GCY,MAM