Product Data Sheet

Product Name: Bz-Nle-Lys-Arg-Arg-AMC
Cat. No.: GA21221

Chemical Properties

Cas. No.	863975-32-0		
Formula	$C_{41}H_{60}N_{12}O_7$	M.Wt	833
Solubility	DMF: 30mg/mL,DMSO: 30mg/mL,DMSO:PBS (pH 7.2) (1:1): 0.5mg/mL,Ethanol: 20mg/mL	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	Bz-Nle-Lys-Arg-Arg-AMC		

Protocol

Cell experiment
[1]:

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	FRET assay was performed to evaluate NS2B-NS3 protease activity and its inhibition by flavonoids. NS2B-NS3 protease in buffer solution (20 mM HEPES, 20 % glycerol and 1 mM CHAPS, pH 9.0) was pre-incubated at 37 °C for 30 min, and the reaction was initiated by adding 100 μM of the substrate Bz-Nle-Lys-Arg-Arg-AMC. The fluorescence was monitored per 15 s in 10 min with a microplate spectrophotometer at 360 nm and 450 nm for excitation and emission, respectively.
Preparation Method	The inhibition assay was performed in 100 μL of buffer containing 20 mM HEPES (pH 9.0), 20 % glycerol, 1 mM CHAPS, 0.1 μM NS2B-NS3 protease and 100 μM substrate with varying concentrations of inhibitors. NS2B-NS3 protease was pre-incubated with inhibitors at 37°C for 30 min, and the reaction was initiated by adding 100 μM of the substrate Bz-Nle-Lys-ArgArg-AMC. After 10 min, the fluorescence was monitored with a microplate spectrophotometer at 360 nm and 450 nm for excitation and emission, respectively. Aprotinin (1 μM) was used as a positive control.
Reaction Conditions	100 μM for 10 min

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References:

[1]: Zou M, Liu H, Li J, Yao X, Chen Y, Ke C, Liu S. Structure-activity relationship of flavonoid bifunctional inhibitors against Zika virus infection. Biochemical pharmacology. 2020 Jul 1;177:113962.

Background

Bz-Nle-Lys-Arg-Arg-AMC is a fluorogenic tetra-peptide substrate for yellow fever virus (YFV) non-structural 3 (NS3), dengue virus (DV) NS2B/3 serine proteases, and Zika virus (ZIKV) NS2B/NS3 serine proteases [1,2,3].

After enzymatic hydrolysis by YNS2B/NS3 serine proteases, Bz-Nle-Lys-Arg-Arg-AMC releases 7-amino-4-methylcoumarin (AMC), whose fluorescence can be used to determine the activity of YNS2B/NS3 serine proteases. AMC exhibits excitation/emission maxima at 340-360/440-460 nm, respectively.

References:

[1]. Ulanday GE, Okamoto K, Morita K. Development and utility of an in vitro, fluorescence-based assay for the discovery of novel compounds against dengue 2 viral protease. Tropical Medicine and Health. 2016 Dec;44:1-0.
[2]. Loehr K, Knox JE, Phong WY, Ma NL, Yin Z, Sampath A, Patel SJ, Wang WL, Chan WL, Rao KR, Wang G. Yellow fever virus NS3 protease: peptide-inhibition studies. Journal of general virology. 2007 Aug;88(8):2223-7.
[3]. Lee H, Ren J, Nocadello S, Rice AJ, Ojeda I, Light S, Minasov G, Vargas J, Nagarathnam D, Anderson WF, Johnson ME. Identification of novel small molecule inhibitors against NS2B/NS3 serine protease from Zika virus. Antiviral research. 2017 Mar 1;139:49-58.

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