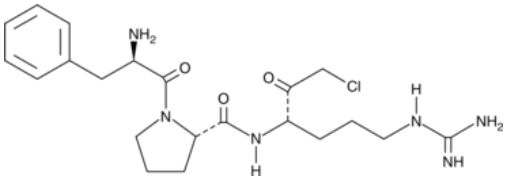
Product Data Sheet

Product Name: PPACK
Cat. No.: GC44671

Chemical Properties

Cas. No.	71142-71-7		
Chemical Name	D-phenylalanyl-N-[(1S)-4-[(aminoiminomethyl)amino]-1-(2-chloroacetyl)butyl]-L-prolinamide, trifluoroacetate salt		
SMILES	O=C([C@H](N)CC1=CC=CC=C1)N2[C@H](C(N[C@H](C(CCl)=O)CCCNC(N)=N)=O)CCC2		
Formula	C21H31ClN6O3	M.Wt	451
Solubility	20mg/mL in ethanol, 33mg/mL in DMSO, or in DMF	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

Kinetic Procedures(Inhibition of Thrombin with PPACK) [1]:

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Preparation method	The thrombin stock solution was diluted in the appropriate buffer, resulting in a 20 mL solution with a concentration of 3-5×10^{-10} M. An aliquot of 980 μL was incubated in a cuvette in the temperature controlled cell compartment of the spectrophotometer to reach thermal equilibrium. Thrombin inhibition was initiated by injecting 10 μL of a thermally equilibrated 1.1 × 10^{-7} to 3.65 × 10^{-7} M PPACK solution and mixing. The reaction mixture was promptly incubated for a time interval between 15 and 200 s, after which 10 μL of a 3-5×10^{-3} M solution of substrate in DMSO was quickly added to measure the remaining enzyme activity. Pseudo-first-order rate constants were calculated by fitting the exponential function to the remaining enzyme activity versus time of inhibition for 10 successive incubation times. The thrombin activity remained constant for the duration of the experiment in the 288-308 K temperature range.
Applications	PPACK is a potent selective and irreversible thrombin inhibitor that inhibits human alpha thrombin with a K_i value of 0.24 nM.
Cell experiment [2]:	
Cell lines	Bovine aortic endothelial cells

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Preparation method	Confluent bovine aortic endothelial cell monolayers were obtained three to five days after plating on the polycarbonate membrane cluster plates. Cells were treated with plasminogen and a plasminogen activator. PPACK were added to the upper compartments. The monolayers were subsequently incubated for two hours in a 5% CO_2 / 95% O_2 atmosphere.
Reaction Conditions	0.2 mM;2h
Applications	Coincubation with PPACK reduced the increase in endothelial permeability induced by plasmin by 59%.
Cell experiment [3]:	
Cell lines	Human osteoarthritis synovial fibroblasts (OASF)
Preparation method	Cell were treated with thrombin (3 U/ml), thrombin plus PPACK (30 nM) for 24 hours.
Reaction Conditions	24h; 30 nM
Applications	Pretreatment of cells with PPACK effectively antagonized the potentiating effect of thrombin on HO-1 expression.
Animal experiment [4]:	

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Animal models	Male rabbits
Preparation method	PPACK was prepared in saline and administered as a bolus dose of 10-100 μg/kg or as an infusion of 0.25-1.0 μg/kg/min. Rabbits were treated with PPACK bolus 1 min either before or after the administration of thrombin or were infused with PPACK commencing 30 min prior to the administration of thrombin and continued for 1 h at a rate of 0.1 mL/min.
Applications	PPACK pretreatment can inhibit thrombine-induced cerebral thromboembolism in rabbits.

References:

[1]. Kovach IM, Kelley P, et,al. Proton bridging in the interactions of thrombin with small inhibitors. Biochemistry. 2009 Aug 4;48(30):7296-304. doi: 10.1021/bi900098s. PMID: 19530705; PMCID: PMC2800789.

[2]. Rabbani LE, Johnstone MT, et,al. PPACK attenuates plasmin-induced changes in endothelial integrity. Thromb Res. 1993 Jun 15;70(6):425-36. doi: 10.1016/0049-3848(93)90085-3. PMID: 8362368.

[3].Liu JF, Hou SM, et,al. Thrombin induces heme oxygenase-1 expression in human synovial fibroblasts through protease-activated receptor signaling pathways. Arthritis Res Ther. 2012 Apr 27;14(2):R91. doi: 10.1186/ar3815. PMID: 22541814; PMCID: PMC3446465.

[4]. Liu JT, Paul W, et,al. Thrombin inhibitors and anti-coagulants on thrombin-induced embolisation in rabbit cranial vasculature. Eur J Pharmacol. 1994 Oct 24;264(2):183-90. doi: 10.1016/0014-2999(94)00464-1. PMID: 7851481.

Background

PPACK is a potent selective and irreversible thrombin inhibitor that inhibits human alpha thrombin

with a K_i value of 0.24 nM. PPACK inhibit the binding of rt-PA to plasma protease inhibitors *in vitro*[1-3]. It forms a covalent bond to the thrombin active site Ser and cross-links with His57 at the active site to form a tetrahedral PPack-thrombin complex. PPACK completely inhibited changes in fibrin degradation products, plasminogen and alpha 2-antiplasmin[4-6].

Coincubation with PPACK(0.2 mM;2h) reduced the increase in endothelial permeability induced by plasmin by 59% in bovine aortic endothelial cells[7]. Heme oxygenase (HO)-1 is a stress-inducible rate-limiting enzyme in heme degradation that confers cytoprotection against oxidative injury. Pretreatment of cells with PPACK(24h; 30 nM) effectively antagonized the potentiating effect of thrombin on HO-1 expression[8].

PPACK(bolus dose of 10-100 μg/kg or infusion of 0.25-1.0 μg/kg/min) pretreatment can inhibit thrombine-induced cerebral thromboembolism in rabbits[9].

References:

[1]. Mohler MA, Refino CJ, et,al. D-Phe-Pro-Arg-chloromethylketone: its potential use in inhibiting the formation of in vitro artifacts in blood collected during tissue-type plasminogen activator thrombolytic therapy. Thromb Haemost. 1986 Oct 21;56(2):160-4. PMID: 2433785.

[2]. Bode W, Mayr I, et,al. The refined 1.9 A crystal structure of human alpha-thrombin: interaction with D-Phe-Pro-Arg chloromethylketone and significance of the Tyr-Pro-Pro-Trp insertion segment. EMBO J. 1989 Nov;8(11):3467-75. doi: 10.1002/j.1460-2075.1989.tb08511.x. PMID: 2583108; PMCID: PMC401503.

[3]. Lyon ME, Fine JS, et,al. D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK): alternative anticoagulant to heparin salts for blood gas and electrolyte specimens. Clin Chem. 1995 Jul;41(7):1038-41. PMID: 7600685.

[4]. Kovach IM, Kelley P, et,al. Proton bridging in the interactions of thrombin with small inhibitors. Biochemistry. 2009 Aug 4;48(30):7296-304. doi: 10.1021/bi900098s. PMID: 19530705; PMCID: PMC2800789.

[5]. Kettner C, Shaw E. D-Phe-Pro-ArgCH2C1-A selective affinity label for thrombin. Thromb Res. 1979;14(6):969-73. doi: 10.1016/0049-3848(79)90014-8. PMID: 473131.

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[6]. Nienaber VL, Mersinger LJ, et,al. Structure-based understanding of ligand affinity using human thrombin as a model system. Biochemistry. 1996 Jul 30;35(30):9690-9. doi: 10.1021/bi952164b. PMID: 8703940.

[7]. Rabbani LE, Johnstone MT, et,al. PPACK attenuates plasmin-induced changes in endothelial integrity. Thromb Res. 1993 Jun 15;70(6):425-36. doi: 10.1016/0049-3848(93)90085-3. PMID: 8362368.

[8].Liu JF, Hou SM, et,al. Thrombin induces heme oxygenase-1 expression in human synovial fibroblasts through protease-activated receptor signaling pathways. Arthritis Res Ther. 2012 Apr 27;14(2):R91. doi: 10.1186/ar3815. PMID: 22541814; PMCID: PMC3446465.

[9]. Liu JT, Paul W, et,al. Thrombin inhibitors and anti-coagulants on thrombin-induced embolisation in rabbit cranial vasculature. Eur J Pharmacol. 1994 Oct 24;264(2):183-90. doi: 10.1016/0014-2999(94)00464-1. PMID: 7851481.

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Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA