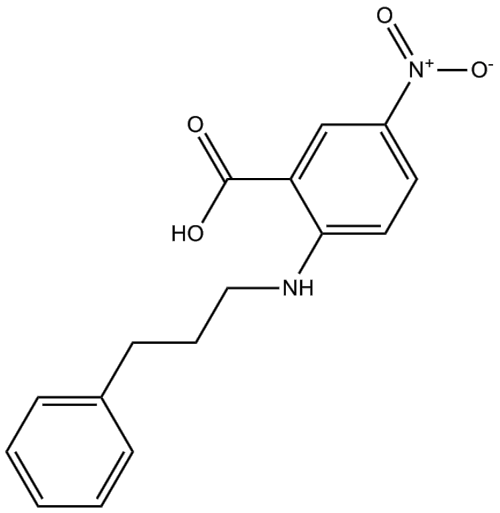
Product Data Sheet

Product Name: NPPB
Cat. No.: GC14268

Chemical Properties

Cas. No.	107254-86-4		
Chemical Name	5-nitro-2-((3-phenylpropyl)amino)benzoic acid		
SMILES	OC(C1=CC([N+]([O-])=O)=CC=C1NCCCC2=CC=CC=C2)=O		
Formula	$C_{16}H_{16}N_2O_4$	M.Wt	300.31
Solubility	≥ 11.05mg/mL in DMSO	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



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Protocol

Cell experiment [1]:

Cell lines	Primary adhesion fibroblasts (AFB)
Preparation Method	Primary peritoneal AFB cells were seeded in 12-well plates at a density of 1.9×10^5/well and allowed to grow from 80 to 90% confluence. The confluent monolayers were scratched with a 10μL pipette tip and then washed with PBS several times to remove cell debris. The culture medium was replaced with medium containing 1% BSA, 1% FBS, and increasing concentrations of NPPB (0, 30, 60, 90, and 120μM). Wound-healing was photographed using an inverted fluorescence microscope to assess the rate of closure. The migration rates were calculated using the equation [(wound area at 0h−wound area at 72h)/wound area at 0h]×100%. The experimental data at 72h were transformed to sigmoidal dose-response curves using non-linear regression analysis to calculate the corresponding IC_{50} values.
Reaction Conditions	30–120μM; 72h
Applications	NPPB inhibited the migration of adhesion fibroblasts (AFB) with an IC_{50} of 56.75μM.

Animal experiment [2]:

Animal models	Albino rabbits

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Preparation Method	Thirty Albino rabbits of both sexes weighting 2.7-3.6kg were divided into five groups. All the animals were anesthetized by 30mg/kg sodium pentobarbital (iv) and ventilated using a respirator. A 5 French high flow multipurpose catheter was placed in the vertebral artery through the femoral artery in each animal. A control angiogram of the basilar artery was performed in each group. Fifteen minutes after the control angiogram, 0.6ml of isotonic saline in Group I, 0.6ml of 1nM ET-1 in Group II were slowly injected into the vertebral arteries of the animals. Thirty minutes after the injection, control angiograms were performed to evaluate the effects of ET-1 and isotonic saline. In the ET-1 plus NPPB treated group (Group III), 0.1ml 20mM NPPB was slowly injected after the control angiogram. 0.6ml, 1nM ET-1 was slowly injected ten minutes after the NPPB administration. The last angiograms were performed thirty minutes after the injection of ET-1 to evaluate the effects of ET-1 in NPPB treated rabbits. In Group IV, the effect of dimethylsulfate (DMS) was tested by a control angiogram thirty minutes after the injection of 0.1ml DMS since NPPB was dissolved in DMS. In Group IV, the effect of NPPB in the normal basilar artery was tested by a control angiogram 30 minutes after the injection of 0.1ml 20mM NPPB. On the subtraction images, the diameter of the basilar artery in pre and post injection angiograms was measured in each group and compared by quantitative analysis software. All animals were sacrificed immediately after the repeat angiography by exsanguination. Basilar arteries were rapidly removed and processed by methods described previously for ultrastructural investigation.
Dosage form	0.1ml; 20mM; intra-arterial injection
Applications	NPPB prevented ET-1-induced vasoconstriction and reversed ET-1-induced histological alterations in the brainstem of rabbits.

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

References:
[1] Zhong J, Qin Z, Yu H, et al. NPPB prevents postoperative peritoneal adhesion formation by blocking volume-activated Cl^- current. *Naunyn Schmiedebergs Arch Pharmacol*. 2020;393(3):501-510.
[2] Dogulu F, Barun S, Emmez H, et al. Effect of a chloride channel inhibitor, 5-nitro-2- (3-phenylpropylamino)-benzoate, on endothelin-1 induced vasoconstriction in rabbit basilar artery. *Turk Neurosurg*. 2009;19(4):380-386.

Background

NPPB is a potent inhibitor of chloride channel with an IC_{50} of 80nM for the short circuit current[1]. NPPB is commonly used in the study of epithelial ion transport mechanisms (such as cystic fibrosis and secretory diarrhea) and cardiovascular physiological regulation[2][3].

In vitro, treatment of human conjunctival fibroblasts (HConFs) with NPPB (100μM; 48h) inhibited proliferation, migration, and cell cycle progression, increased apoptosis, and reduced collagen I and fibronectin expression[4]. NPPB (30–120μM; 72h) inhibited the migration of adhesion fibroblasts (AFB) with an IC_{50} of 56.75μM, reduced cell proliferation in a concentration- and time-dependent manner, blocked volume-activated chloride currents (ICl, vol), and inhibited the regulatory volume decrease (RVD)[5].

In vivo, NPPB (20 and 40mg/kg; i.p.; 1–7h) increased mechanical and thermal hyperalgesia in crush injury-induced neuropathic pain in rats[6]. NPPB (0.1ml; 20mM; intra-arterial injection) prevented ET-1-induced vasoconstriction and reversed ET-1-induced histological alterations in the brainstem of rabbits[7].

References:
[1] Wangemann P, Wittner M, Di Stefano A, et al. Cl(-)-channel blockers in the thick ascending limb of the loop of Henle. Structure activity relationship. *Pflugers Arch*. 1986;407 Suppl 2:S128-S141.
[2] Lin WY, Sohma Y, Hwang TC. Synergistic Potentiation of Cystic Fibrosis Transmembrane Conductance Regulator Gating by Two Chemically Distinct Potentiators, Ivacaftor (VX-770) and 5-Nitro-2-(3-Phenylpropylamino) Benzoate. *Mol Pharmacol*. 2016;90(3):275-285.

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[3] Diaz RJ, Losito VA, Mao GD, Ford MK, Backx PH, Wilson GJ. Chloride channel inhibition blocks the protection of ischemic preconditioning and hypo-osmotic stress in rabbit ventricular myocardium. *Circ Res*. 1999;84(7):763-775.
[4] Sun L, Dong Y, Zhao J, et al. NPPB modulates apoptosis, proliferation, migration and extracellular matrix synthesis of conjunctival fibroblasts by inhibiting PI3K/AKT signaling. *Int J Mol Med*. 2018;41(3):1331-1338.
[5] Zhong J, Qin Z, Yu H, et al. NPPB prevents postoperative peritoneal adhesion formation by blocking volume-activated Cl^- current. *Naunyn Schmiedebergs Arch Pharmacol*. 2020;393(3):501-510.
[6] Ramteke VD, Tandan SK, Kumar D, Aruna Devi R, Shukla MK, Ravi Prakash V. Increased hyperalgesia by 5-nitro-2, 3-(phenylpropylamino)-benzoic acid (NPPB), a chloride channel blocker in crush injury-induced neuropathic pain in rats. *Pharmacol Biochem Behav*. 2009;91(3):417-422.
[7] Dogulu F, Barun S, Emmez H, et al. Effect of a chloride channel inhibitor, 5-nitro-2- (3-phenylpropylamino)-benzoate, on endothelin-1 induced vasoconstriction in rabbit basilar artery. *Turk Neurosurg*. 2009;19(4):380-386.