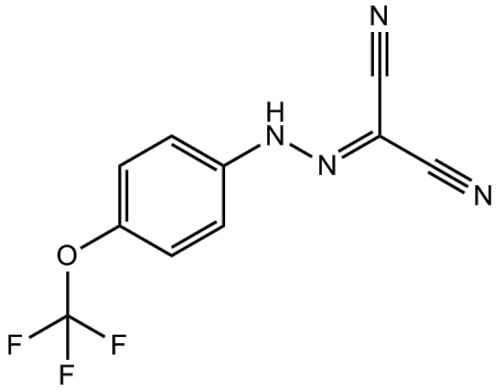
Product Data Sheet

Product Name: FCCP
Cat. No.: GC14328

Chemical Properties

Cas. No.	370-86-5		
Chemical Name	(4-(trifluoromethoxy)phenyl)carbonohydrazonoyl dicyanide		
SMILES	FC(F)(OC1=CC=C(C=C1)N/N=C(C#N)/C#N)F		
Formula	$C_{10}H_5F_3N_4O$	M.Wt	254.17
Solubility	≥ 56.6 mg/mL in DMSO with ultrasonic, ≥ 25 mg/mL in EtOH with ultrasonic	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

Cell experiment [1]:

Product Data Sheet

Cell lines	HuH6,Hep3B, HepG2, HLE cells (human hepatocellular carcinoma cell line)
Preparation Method	HuH6, Hep3B, HepG2 and HLE cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% FBS at 37°C, 5% CO_2. Cells were seeded in 96-well plates and treated with 1μM Gemcitabine alone or in combination with FCCP (4-trifluoro-methoxy-phenyl-hydrazone) for 16h (mitochondrial superoxide assay) or 48h (cell growth inhibition assay). After treatment, mitochondrial superoxide was measured by MitoSox Red (Catalog No.GC68230) and cell growth was measured by MTT (Catalog No.GC14731) assay.
Reaction Conditions	1μM; 16h or 48h
Applications	FCCP co-treatment with Gemcitabine induced resistance to Gemcitabine-induced cell growth inhibition in HuH6, Hep3B, HepG2 and HLE cells. FCCP co-treatment with Gemcitabine prevented Gemcitabine-induced mitochondrial superoxide generation in HuH6, Hep3B, HepG2 and HLE cells.
Animal experiment [2]:	
Animal models	Wild-type C57BL/6 mice, PINK1-deficient ($PINK1^{-/-}$) mice
Preparation Method	Mice were intraperitoneally administered 1mg/kg FCCP in saline twice daily. The final dose was given 1 hour prior to tissue collection. Hearts were excised, retrograde perfused via aorta with Krebs-Henseleit buffer for 5min equilibration then with 100nM FCCP in perfusion buffer for 5min or 15min. Ventricular tissue was used for mitochondrial isolation, Western blot of Parkin, PINK1, ubiquitin, LC3II, Tom20, complex markers, and GAPDH.

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

Dosage form	1mg/kg; i.p.; twice daily for one day 100nM; cardiac perfusion for 5min or 15min
Applications	FCCP administration increased Parkin levels in the mitochondrial fraction of wild-type and PINK1$^{-/-}$ hearts. FCCP perfusion did not cause endogenous PINK1 accumulation at mitochondria in wild-type hearts at 5min or 15min. FCCP in vivo increased Parkin translocation to mitochondria in wild-type and PINK1$^{-/-}$ hearts, increased ubiquitination of mitochondrial proteins, and increased association of LC3II with mitochondria in wild-type and PINK1$^{-/-}$ hearts.

References:

[1] Yu G, Liu J, Xu K, et al. Uncoupling protein 2 mediates resistance to gemcitabine-induced apoptosis in hepatocellular carcinoma cell lines. Biosci Rep. 2015;35(4):e00231.

[2] Kubli DA, Cortez MQ, Moyzis AG, et al. PINK1 Is Dispensable for Mitochondrial Recruitment of Parkin and Activation of Mitophagy in Cardiac Myocytes. PLoS ONE. 2015 Jun 25;10(6):e0130707.

Background

FCCP is a highly lipophilic mitochondrial uncoupler. FCCP acts as a protonophore across the inner mitochondrial membrane to transport protons, dissipate mitochondrial membrane potential and pH gradient, uncouple electron transport from ATP synthesis, increase oxygen consumption while inhibiting ATP production, and trigger energy stress by reducing mitochondrial ATP synthesis, affecting AMPK and other energy sensing nodes as well as HIF signaling. FCCP can be used for research on mitochondrial function, cellular energy metabolism, hypoxia signaling and tumor metabolism[1-4].

In vitro, co-treatment of HuH6, Hep3B, HepG2 and HLE cells with 1μM FCCP and Gemcitabine for 16-48 hours inhibited Gemcitabine-induced mitochondrial superoxide generation and induced resistance to Gemcitabine-inhibited cell growth[5]. Treatment of niclosamide-pretreated HEK293T

cells with 10μM FCCP for 1 hour reduced maximal cellular respiration rate[6]. Treatment of 4T1 or B16 cells with 0.5μM FCCP for 30 minutes induced peak uncoupled oxygen consumption rate[7].

In vivo, twice-daily intraperitoneal injection of 1mg/kg FCCP for 1 day in C57BL/6 mice and PINK1-deficient ($PINK1^{-/-}$) mice caused Parkin translocation to cardiac mitochondria, accompanied by increased ubiquitination of mitochondrial proteins and increased association of autophagy protein LC3II with mitochondria. Retrograde aortic perfusion of wild-type and $PINK1^{-/-}$ mouse hearts with 100nM FCCP for 5-15 minutes caused rapid Parkin accumulation in the cardiac mitochondrial fraction without causing endogenous PINK1 accumulation at mitochondria[8].

References:

[1] Galkina KV, Finkelberg JM, Markova OV, et al. Protonophore FCCP provides fitness advantage to PDR-deficient yeast cells. J Bioenerg Biomembr. 2020 Oct;52(5):383-395.

[2] Park KS, Jo I, Pak K, et al. FCCP depolarizes plasma membrane potential by activating proton and Na+ currents in bovine aortic endothelial cells. Pflugers Arch. 2002 Jan;443(3):344-52.

[3] Jensen JR, Rehder V. FCCP releases Ca2+ from a non-mitochondrial store in an identified Helisoma neuron. Brain Res. 1991 Jun 14;551(1-2):311-4.

[4] Brennan JP, Southworth R, Medina RA, et al. Mitochondrial uncoupling, with low concentration FCCP, induces ROS-dependent cardioprotection independent of KATP channel activation. Cardiovasc Res. 2006 Nov 1;72(2):313-21.

[5] Yu G, Liu J, Xu K, et al. Uncoupling protein 2 mediates resistance to gemcitabine-induced apoptosis in hepatocellular carcinoma cell lines. Biosci Rep. 2015;35(4):e00231.

[6] Ng MY, Song ZJ, Tan CH, et al. Structural investigations on the mitochondrial uncouplers niclosamide and FCCP. FEBS Open Bio. 2024;14(6):1057-1071.

[7] Jiang X, Fan Z, Zhang Z, et al. Tumor metabolome remolded by low dose mitochondrial uncoupler elicites robust CD8+ T cell response. Cell Death Discov. 2025;11:291.

[8] Kubli DA, Cortez MQ, Moyzis AG, et al. PINK1 Is Dispensable for Mitochondrial Recruitment of Parkin and Activation of Mitophagy in Cardiac Myocytes. PLoS ONE. 2015 Jun 25;10(6):e0130707.

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