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产品名称: 羰基氰酯-3-氯苯基脒  
产品别名: CCCP ; Carbonyl cyanide 3-chlorophenylhydrazone;  
Carbonyl Cyanide m-Chlorophenylhydrazone

生物活性:				
Description	CCCP is an oxidative phosphorylation uncoupler. CCCP induces activation of PINK1 leading to Parkin Ser65 phosphorylation.			
IC <sub>50</sub> & Target	STING[1] IFN-β[1]			
In Vitro	CCCP inhibits IFN-β production induced by various types of the STING pathway activators. CCCP suppresses the phosphorylation of STING, TBK1, and IRF3 via disrupting the association of STING and TBK1. CCCP inhibits activation of STING and its downstream signaling molecules, TBK1 and IRF3, but not STING translocation to the perinuclear region. CCCP impairs the interaction between STING and TBK1 and concomitantly triggers mitochondria fission. Importantly, the knockout of the crucial mitochondria fission regulator Drp1 restored the STING activity, indicating that CCCP down-modulates the STING pathway through DRP1-mediated mitochondria fragmentation. The protonophore CCCP that disrupts membrane potential suppresses the DMXAA-triggered STING signaling pathway. CCCP drastically suppresses the production of IFN-β in DMXAA-treated RAW264.7 cells and MEFs[1].			
In Vivo	The same dosage of 3 mg/kg.bw each of CCCP and PPEF is used. In both the cases 1 log reduction is observed in the bacterial load. However, when 3 mg/kg.bw of PPEF is used in combination with 3 mg/kg.bw of CCCP, 6 log <sub>10</sub> reduction is observed in the bacterial count. The developed model validates the enhanced antibacterial activity of combination therapy[2]. <sup>99m</sup> Tc-MIBI signals in the hearts of SD rats administered CCCP (4 mg/kg intraperitoneally) or vehicle is also measured. <sup>99m</sup> Tc-MIBI signals decrease in rat hearts administered CCCP, and the ATP content, as measured by <sup>31</sup> P magnetic resonance spectroscopy, decreased simultaneously. To investigate whether CCCP decreased the <sup>99m</sup> Tc-MIBI signals in rats, we analyzed the radioisotope activity of excised heart tissue from rats administered CCCP. At 180 min after <sup>99m</sup> Tc-MIBI injection, the <sup>99m</sup> Tc-MIBI signals from the hearts in the CCCP group are significantly lower than those in the vehicle group[3].			
<b>In Vitro:</b> <b>DMSO : ≥ 100 mg/mL (488.71 mM)</b>  * "≥" means soluble, but saturation unknown.				
Preparing Stock Solutions	<div>Solvent / Mass / Concentration</div>	1 mg	5 mg	10 mg
	1 mM	4.8871 mL	24.4355 mL	48.8711 mL
	5 mM	0.9774 mL	4.8871 mL	9.7742 mL
	10 mM	0.4887 mL	2.4436 mL	4.8871 mL
<b>*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液。一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。</b>  <b>储备液的保存方式和期限</b> -80℃, 6 months; -20℃, 1 month。 -80℃ 储存时，请在 6 个月内使用，-20℃ 储存时，请在 1 个月内使用。				
<b>In Vivo:</b>				



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<b>Solvent&amp;Solubility</b>	<p>请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：</p> <p>——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用；以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶</p> <p>1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline</p> <p>Solubility: <math>\geq 2.5</math> mg/mL (12.22 mM); Clear solution</p> <p>此方案可获得 <math>\geq 2.5</math> mg/mL (12.22 mM, 饱和度未知) 的澄清溶液。</p> <p>以 1 mL 工作液为例，取 100 <math>\mu</math>L 25.0 mg/mL 的澄清 DMSO 储备液加到 400 <math>\mu</math>L PEG300 中，混合均匀向上述体系中加入 50 <math>\mu</math>L Tween-80，混合均匀；然后继续加入 450 <math>\mu</math>L 生理盐水定容至 1 mL。</p>
<b>References</b>	<p>[1]. Kwon D, et al. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) suppresses STING-mediated DNA sensing pathway through inducing mitochondrial fission. <i>Biochem Biophys Res Commun</i>. 2017 Aug 30. pii: S0006-291X(17)31704-7.</p> <p>[2]. Sinha D, et al. Synergistic efficacy of Bisbenzimidazole and Carbonyl Cyanide 3-Chlorophenylhydrazone combination against MDR bacterial strains. <i>Sci Rep</i>. 2017 Mar 17;7:44419.</p> <p>[3]. Kawamoto A, et al. Measurement of technetium-99m sestamibi signals in rats administered a mitochondrial uncoupler and in a rat model of heart failure. <i>PLoS One</i>. 2015 Jan 16;10(1):e0117091.</p> <p>[4]. Kondapalli C, et al. PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. <i>Open Biol</i>. 2012 May;2(5):120080.</p>
<b>实验参考：</b>	
<b>Cell Assay</b>	<p>MEFs (<math>5 \times 10^5</math>), Raw264.7 cells (<math>1 \times 10^6</math>), and HeLa cells stable expressing STING (<math>1.5 \times 10^5</math>) are stimulated with DMXAA (100 <math>\mu</math>g/mL) for 2 or 3 h, or transfected with c-di-GMP (5 <math>\mu</math>M), cGAMP (5 <math>\mu</math>g/mL), or poly (dA:dT) (2 <math>\mu</math>g/mL) for 6 h. CCCP (50 <math>\mu</math>M) is co-treated with DMXAA (100 <math>\mu</math>g/mL), or treated for the last 5 h in case of treatment of c-di-GMP or poly (dA:dT)[1].</p>
<b>Animal Administration</b>	<p>Mice[2]</p> <p>Female Balb/c mice n=6, per dosing group weighing 20-25 g are rendered neutropenic with 2 intraperitoneal injections of cyclophosphamide 150 mg/kg.bw and 100 mg/kg.bw on 4 days and 1 day prior to bacterial infection. 0.1 mL of the <math>10^6</math> CFU/mL bacterial suspension is injected into right posterior thigh muscle. After 2 h post-infection mice are treated with PPEF (3 mg/kg.bw), CCCP (3 mg/kg.bw) and in combination PPEF+CCCP (3 mg/kg.bw+3 mg/kg.bw) dissolved in 0.1 mL sterile water by single bolus intravenous injection. Twenty-four hours after antibacterial administration, the mice are humanely sacrificed. Right thigh muscles from each mouse are aseptically collected, homogenized and serially diluted and processed for quantitative cultures.</p> <p>Rats[3]</p> <p>Rats are randomly divided into three groups. One group is euthanized 15 min after a dose of 12.5 MBq (337.8 <math>\mu</math>Ci) <math>^{99m}</math>Tc-MIBI injection (n=6). The other two groups are administered 4 mg/kg CCCP (CCCP group; n=7) or vehicle (vehicle group; n=7) by intraperitoneal (i.p.) injection 90 min after the same dose of <math>^{99m}</math>Tc-MIBI injection and are euthanized after an additional 90 min (180 min after the <math>^{99m}</math>Tc-MIBI injection). Hearts are excised and weighed, and radioactivity is measured between 110 and 170 keV with an auto-well gamma counter. <math>^{99m}</math>Tc-MIBI signals are corrected for physical decay (half-life=6 h).</p>



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## References

- [1]. Kwon D, et al. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) suppresses STING-mediated DNA sensing pathway through inducing mitochondrial fission. *Biochem Biophys Res Commun.* 2017 Aug 30. pii: S0006-291X(17)31704-7.
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