CCG 203769

Cat. No.: HY-U00431

CAS No.: 410074-60-1

Molecular Formula: $C_8H_{14}N_2O_2S$

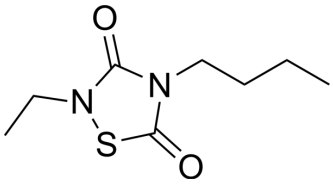
Molecular Weight: 202.27

Target: RGS Protein

Pathway: GPCR/G Protein

Storage:

Pure form	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 62.5 mg/mL (308.99 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	4.9439 mL	24.7194 mL	49.4389 mL
5 mM	0.9888 mL	4.9439 mL	9.8878 mL
10 mM	0.4944 mL	2.4719 mL	4.9439 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (10.28 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (10.28 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (10.28 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

CCG 203769 is a selective G protein signaling (RGS4) inhibitor, which blocks the RGS4-$G\alpha_o$ protein-protein interaction in vitro with an IC_{50} of 17 nM.

IC_{50} & Target

RGS4 17 nM (IC_{50})	RGS19 140 nM (IC_{50})	RGS16 6 μM (IC_{50})	RGS8 79 μM (IC_{50})
GSK3β 5.4 μM (IC_{50})			

In Vitro	CCG 203769 also displays dramatic selectivity (8- to >5000-fold) for RGS4 over other RGS proteins. CCG 203769 inhibits RGS19 with an IC_{50} of 140 nM (8-fold selective for RGS4) and 6 μM for RGS16 (350-fold selective for RGS4). The closely related RGS8 is very weakly inhibited (IC_{50}>60 μM) providing >4500-fold selectivity for RGS4. CCG 203769 inhibits GSK-3β with an IC_{50} value of 5 μM. CCG 203769 does not inhibit the cysteine protease papain at 100 μM. CCG 203769 does not inhibit RGS7, which lacks cysteines in the RGS domain. CCG 203769 inhibits RGS/$G\alpha_o$ binding in an RGS-selective manner. CCG 203769 enhances $G\alpha_q$-dependent cellular Ca^{2+} signaling in an RGS4-dependent manner. CCG 203769 also blocks the GTPase accelerating protein (GAP) activity of RGS4. In single-turnover and steady-state GTPase experiments with $G\alpha_o$ and $G\alpha_{i1}$, the rate of GTP hydrolysis is strongly stimulated by RGS4, and this effect is inhibited by CCG 203769 with an IC_{50}<1 μM[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	To determine whether this genetic disruption of RGS4 function can be replicated pharmacologically, CCG 203769 is tested for effects on Carbamoylcholine chloride-mediated bradycardia in conscious, unrestrained rats. Carbamoylcholine chloride (0.1 mg/kg, IP) produces a modest decrease in heart rate compared to that of a saline vehicle control. CCG 203769 (10 mg/kg, IV) has no significant effect upon heart rate when given alone. However, CCG 203769, administered immediately prior to Carbamoylcholine chloride, significantly potentiates the bradycardic effect ($p < 0.05$). Given the functional role of RGS4 in Parkinson's disease models, CCG 203769 is tested in a pharmacologic model of D2 antagonist-induced bradykinesia. Raclopride administration in rats causes increased hang time in the bar test, which is rapidly reversed by doses of CCG 203769 ranging from 0.1 to 10 mg/kg. The lowest dose, 0.01 mg/kg has no effect, while 0.1 mg/kg produces a submaximal effect. The higher doses, 1 and 10 mg/kg, produce equivalent effects. Similarly, the raclopride-induced paw drag in mice is reversed by 0.1-10 mg/kg CCG 203769[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Steady-state hydrolysis of unlabeled GTP is measured using malachite green in a receptor-independent assay utilizing a mutant $G\alpha_{i1}$ (R178M, A326S). These mutations facilitate the release of GDP from the enzyme making the GTP hydrolysis step rate-limiting. GTP hydrolysis is measured by mixing 6 μM mutant $G\alpha_i$ with 300 μM GTP in 100 μL in 96-well plates in the presence or absence of 200 nM RGS4 and CCG-203769 or DMSO (vehicle control). All assay components are diluted in a buffer comprising 50 mM HEPES at pH 7.4, 100 mM NaCl, 0.01% Lubrol, 5 mM MgCl, and 10 μg/mL BSA. The reaction is allowed to proceed for 2 h at room temperature and then is quenched with 60 μL of an HCl/malachite green dye solution. Immediately after the addition of malachite green, 10 μL of 32% w/v sodium citrate is added as a colorimetric stabilizer, followed by incubation at room temperature for 20 min. Released inorganic phosphate is measured as an increase in absorbance (A_{630}) from the complex of phosphate with malachite green. Background control samples lacking Gα are used to determine the rate of nonenzymatic GTP hydrolysis which is subtracted[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Mice[1] Young male (20-25 g; 8-9 weeks) C57BL/6J mice are used. Akinesia and bradykinesia are assessed 30 min after Raclopride, mice receive either DMSO or CCG-203769 (0.1-10 mg/kg, i.p.). Behavior is assessed 20 or 90 min after DMSO or CCG-203769. Rats[1] Adult Sprauge-Dawley rats receive CCG-203769 (10 mg/kg, i.v.) or saline (by i.v. infusion through the indwelling venous catheter over 30 s) while freely moving in their homecage. One minute later, saline or 0.1 mg/kg Carbamoylcholine chloride (i.p.) is administered. Before and after i.v. infusions, catheters are flushed with approximately 0.5 mL of heparinized saline (50 U/mL) to check catheter patency and flush treatments from the dead space in the catheter. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Blazer LL, et al. Selectivity and anti-Parkinson's potential of thiadiazolidinone RGS4 inhibitors. ACS Chem Neurosci. 2015 Jun 17;6(6):911-9.

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

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