

Product Data Sheet

Product Name: Trimethoprim

Cat. No.: GC10137

Chemical Properties

Cas. No. 738-70-5

Chemical Name 5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine

SMILES COC1=CC(=CC(=C1OC)OC)CC2=CN=C(N=C2N)N

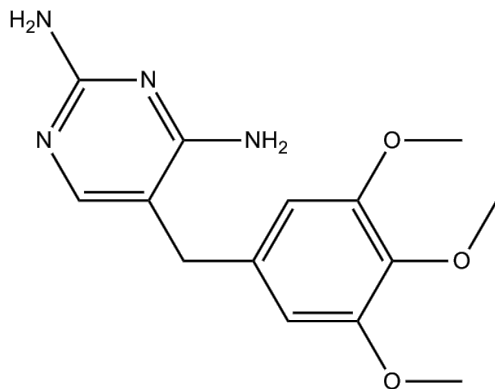
Formula $C_{14}H_{18}N_4O_3$ M.Wt 290.32

Solubility $\geq 12.25\text{mg/mL}$ in DMSO Storage Store at 2-8°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

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

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Cell experiment [1]:	
Cell lines	HCT116, RKO, DLD1 cells (human colorectal carcinoma cell line), MCF7 cells (human breast adenocarcinoma cell line), SUM159 cells (human triple-negative breast cancer cell line), 4T1 cells, PyMT cells (mouse mammary tumor cell line)
Preparation Method	Cells were maintained in DMEM or RPMI 1640 supplemented with 10% FBS at 37°C, 5% CO ₂ . Cells were treated with Trimethoprim at 0.1-4mM for 48h. After treatment, cell viability was measured by CCK-8 and trypan blue/DAPI staining, apoptosis by Annexin V/PI flow cytometry, cell cycle by nocodazole synchronization and FACS, migration by transwell assay, and Snail/E-cadherin/vimentin/cyclin D1/CDK4/CDK6/p-Rb protein levels by immunoblotting.
Reaction Conditions	0.1-4mM; 48h
Applications	Trimethoprim dose-dependently reduced cell viability without inducing apoptosis or direct cell death in HCT116 and RKO cells. Trimethoprim decreased Snail protein levels and increased G1 phase cell fraction with reduced cyclin D1, CDK4, CDK6 and phospho-Rb expressions. Trimethoprim increased E-cadherin and decreased vimentin, N-cadherin and fibronectin levels, and inhibited cell migration in transwell assays.
Animal experiment [2]:	
Animal models	White mice

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Preparation Method	Mice were intraperitoneally infected with 0.5mL of <i>Toxoplasma gondii</i> RH strain suspension ($\sim 7 \times 10^3$ protozoans/mL). Simultaneously with infection, mice received daily per os treatment with Trimethoprim 2mg alone, sulphamethoxazole 2mg alone, or Trimethoprim 2mg combined with sulphamethoxazole 1mg for 10 or 20 days. Survival was recorded up to day 20 or day 30 post-inoculation. For carrier state testing, brain suspensions from surviving mice were injected intraperitoneally into four recipient mice, and peritoneal exudate or brain tissue was examined microscopically or by further mouse passage.
Dosage form	2mg; p.o.; 10 or 20 days
Applications	Trimethoprim 2mg alone showed no effect on survival of infected mice. Trimethoprim 2mg combined with sulphamethoxazole 1mg significantly prolonged survival compared with sulphamethoxazole 1mg alone.
References: [1] Ren BX, Li Y, Li HM, et al. The antibiotic drug trimethoprim suppresses tumour growth and metastasis via targeting Snail. <i>Br J Pharmacol</i> . 2022;179(11):2659-2677. [2] Sander J, Midtvedt T. The effect of trimethoprim on acute experimental toxoplasmosis in mice. <i>Acta Pathol Microbiol Scand B</i> . 1970;78(6):664-8.	

Background

Trimethoprim is a bacteriostatic antibiotic inhibiting dihydrofolate reductase (DHFR) enzyme activity. Trimethoprim selectively and reversibly inhibits bacterial DHFR, blocks the conversion of dihydrofolate to tetrahydrofolate, and interferes with bacterial nucleic acid and protein biosynthesis to exert antibacterial effects. Trimethoprim has antibacterial activity against susceptible Gram-positive aerobic cocci and Gram-negative aerobic bacilli such as *Escherichia coli*, *Proteus mirabilis*,

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Klebsiella pneumoniae, *Enterobacter* species and *Staphylococcus saprophyticus*. Trimethoprim is often used in combination with sulphamethoxazole to enhance antibacterial effects. Trimethoprim can be used for research and treatment of urinary tract infections, traveler's diarrhea, acute exacerbations of chronic bronchitis and *Pneumocystis jirovecii* pneumonia^[1-4].

In vitro, treatment of peripheral blood lymphocytes with 25-50µg/mL Trimethoprim for 24-48 hours increased micronucleus frequency. Treatment of F2408 and 5RP7 cells with 5-200µM Trimethoprim for 24-72 hours inhibited cell proliferation and decreased mitochondrial activity^[5]. Treatment of HCT116, RKO, DLD1, MCF7, 4T1, SUM159 and PyMT cells with 0.1-4mM Trimethoprim for 48 hours reduced cell viability and induced G1 phase arrest, downregulated cyclin D1, CDK4, CDK6 and phospho-Rb protein expression, decreased Snail protein level and promoted ubiquitination degradation, weakened Snail binding to CBP/p300 and Snail acetylation, elevated Snail phosphorylation level, increased E-cadherin and decreased vimentin, N-cadherin and fibronectin expression, and inhibited cell migration^[6]. Treatment of foreskin fibroblasts co-cultured with *Toxoplasma gondii* 2F strain with 46µM Trimethoprim for 5 days inhibited tachyzoite growth^[7].

In vivo, feeding white mice intraperitoneally inoculated with *Toxoplasma gondii* RH strain with food containing 2mg Trimethoprim and 1mg sulphamethoxazole for 20 days prolonged survival days of infected mice^[8]. Intravenous injection of 25mg/kg Trimethoprim into Sprague-Dawley rats via jugular vein 15 minutes before intravenous injection of 1mg/kg emtricitabine decreased emtricitabine clearance, prolonged emtricitabine elimination half-life, increased apparent volume of distribution, and decreased renal excretion rate of emtricitabine^[9]. Administration of 35mg/kg-70mg/kg Trimethoprim via drinking water to Swiss Webster mice intraperitoneally inoculated with *Toxoplasma gondii* RH strain for 13 days failed to eradicate *Toxoplasma* and did not improve mouse survival^[10].

References:

- [1] Gleckman R, Blagg N, Joubert DW. Trimethoprim: mechanisms of action, antimicrobial activity, bacterial resistance, pharmacokinetics, adverse reactions, and therapeutic indications. *Pharmacotherapy*. 1981 Jul-Aug;1(1):28-38.
- [2] Rich G, Mee B. Trimethoprim. *Med J Aust*. 1985 Mar 18;142(6):350-2.
- [3] Brogden RN, Carmine AA, Heel RC, et al. Trimethoprim: a review of its antibacterial activity,

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pharmacokinetics and therapeutic use in urinary tract infections. *Drugs*. 1982 Jun;23(6):405-30.

[4] Long MJC, Pan Y, Lin HC, et al. Cell Compatible Trimethoprim-Decorated Iron Oxide Nanoparticles Bind Dihydrofolate Reductase for Magnetically Modulating Focal Adhesion of Mammalian Cells. *J Am Chem Soc*. 2011 Jun 10;133(26):10006-10009.

[5] Guzel Bayülken D, Bostancioglu R B, Koparal A T, et al. Assessment of in vitro cytotoxic and genotoxic activities of some trimethoprim conjugates. *Cytotechnology*. 2018 Jun;70(3):1051-1059.

[6] Ren BX, Li Y, Li HM, et al. The antibiotic drug trimethoprim suppresses tumour growth and metastasis via targeting Snail. *Br J Pharmacol*. 2022 May;179(11):2659-2677.

[7] Hencken CP, Jones-Brando L, Bordon C, et al. Thiazole, Oxadiazole, and Carboxamide Derivatives of Artemisinin are Highly Selective and Potent Inhibitors of *Toxoplasma gondii*. *J Med Chem*. 2010 May 13;53(9):3594-3601.

[8] Sander J, Midtvedt T. The effect of trimethoprim on acute experimental toxoplasmosis in mice. *Acta Pathol Microbiol Scand B*. 1970;78(6):664-668.

[9] Nakatani-Freshwater T, Taft DR. Renal Excretion of Emtricitabine II. Effect of Trimethoprim on Emtricitabine Excretion: In Vitro and In Vivo Studies. *J Pharm Sci*. 2008 Dec;97(12):5411-5420.

[10] Dumas JL, Pizzolato G, Pechère JC. Evaluation of trimethoprim and sulphamethoxazole as monotherapy or in combination in the management of toxoplasmosis in murine models. *Int J Antimicrob Agents*. 1999 Jan;13(1):35-39.

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