

Product Data Sheet

Product Name: WIN 18446

Cat. No.: GC10438

Chemical Properties

Cas. No. 1477-57-2

Chemical Name N,N'-(octane-1,8-diyl)bis(2,2-dichloroacetamide)

SMILES ClC(C(NCCCCCCCCNC(C(Cl)Cl)=O)=O)Cl

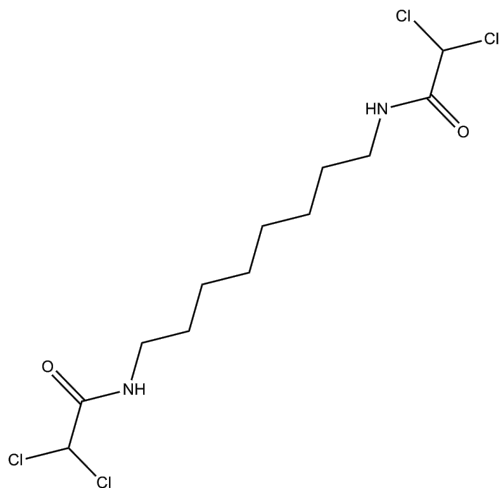
Formula $C_{12}H_{20}Cl_4N_2O_2$ M.Wt 366.11

Solubility $\geq 11.4\text{mg/mL}$ 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



Protocol

Kinase experiment [1,2]:

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Human ALDH1A2 cDNA was cloned from human testis RNA to generate human ALDH1A2 enzyme. ALDH1A2 enzyme was incubated in assay buffer containing 20mM Hepes, 150mM KCl and 1mM EDTA^[1]. 0.1μM, 1μM or 3μM of WIN 18446 was dissolved in DMSO (concentration of DMSO was less than 1%)^[1]. Preparation Method Dissolved WIN 18446 was added to assay buffer with 2.5, 20.5, 50, 100, 500, 1000 and 2000ng/ml of retinol dissolved in ethanol for 20, 40, 60, 80mins as treatment group^[2]. Concentration of retinoic acid were determined by HPLC. This protocol only provides a guideline, and should be modified according to your specific needs.

Reaction Conditions WIN 18446: 0.1μM, 1μM or 3μM; Retinol: 2.5, 20.5, 50, 100, 500, 1000 and 2000ng/ml; 20, 40, 60, 80mins

Applications After 80mins treatment with WIN 18446 (0.1μM, 1μM or 3μM) ALDH1A2 enzyme retained only 2-3% of its activity. As the incubation time increased until 80mins, the rate of retinoic acid production reached saturation with increased concentration of retinal.

Animal experiment [3]:

Animal models C57BL/6 male mice

Preparation Method C57BL/6 male mice (breeding age, n=60) were fed WIN 18446 (2mg/g diet) with AIN9M (control diet) for 3, 7, 14, 21, and 28 days^[3]. Five of the sixty mice per time point were euthanized and testes were collected. After 28 days, the rest of mice were fed with AIN9M (control diet) only for recovery assay for 42 and 56 days (up to 8 weeks) ^[3]. Five recovery group mice were euthanized per time point and testes were collected. Control group mice (n=15) were fed with AIN9M (control diet) only^[3]. Testes specimens were fixed in Davidson's fixative for 24–48h, then transferred to 70% ethanol for testicular histology analysis^[3]. This protocol only provides a guideline, and should be modified according to your specific needs.

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Dosage form	WIN 18446: 2mg/g diet for 3, 7, 14, 21, 28, 42 and 56 days; AIN9M: control diet for 3, 7, 14, 21, 28, 42 and 56 days
Applications	Testes weights from WIN 18446 (2mg/g diet) treated mice group were significantly reduced. Testes weights from recovery group (AIN9M control diet only) still remained smaller compared to control groups. After 28 days treatment of WIN 18446 (2mg/g diet), most testicular tubules exhibited severe epithelial disruption, characterized by the loss of germ cells and the presence of frequent multinucleated cells.

[1] Paik J, Haenisch M, Muller CH, Goldstein AS, Arnold S, Isoherranen N, Brabb T, Treuting PM, Amory JK. Inhibition of retinoic acid biosynthesis by the bisdichloroacetyldiamine WIN 18,446 markedly suppresses spermatogenesis and alters retinoid metabolism in mice. *Journal of Biological Chemistry*. 2014 May 23;289(21):15104-17.

[2] Hartmann S, Froescheis O, Ringenbach F, Wyss R, Bucheli F, Bischof S, Bausch J, Wiegand UW. Determination of retinol and retinyl esters in human plasma by high-performance liquid chromatography with automated column switching and ultraviolet detection. *Journal of Chromatography B: Biomedical Sciences and Applications*. 2001 Feb 25;751(2):265-75.

[3] Paik J, Snyder JM, Kim A, Haenisch M, Fogassy K, Amory JK. Kinetics of the inhibition and recovery of spermatogenesis induced by treatment with WIN 18,446, a male contraceptive, in mice. *Andrology*. 2024.

Background

WIN 18446 (Fertilysin) is a potent inhibitor of aldehyde dehydrogenase 1A2 (ALDH1A2) enzyme with an IC_{50} value of $0.3\mu M$ ^[1]. WIN 18446 has been made as a candidate for male contraception studies^[2]. ALDH1a2 is an enzyme involved in retinoic acid biosynthesis^[3]. Therefore, inhibiting ALDH1A2 disrupts retinoic acid synthesis, leading suppression of spermatogenesis^[3].

In vitro, purified ALDH1A2 enzyme was obtained from cloned human ALDH1A2 testis cDNA^[4]. $0.1\mu M$, $1\mu M$ or $3\mu M$ of WIN 18446 was added to ALDH1A2 enzyme alone with 2.5, 20.5, 50, 100, 500, 1000 and 2000ng/ml of retinol for 20, 40, 60, 80mins. After 80mins treatment with WIN 18446 ($0.1\mu M$, $1\mu M$ or $3\mu M$) ALDH1A2 enzyme retained only 2-3% of its activity^[4,5]. As the incubation time

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increased until 80mins, the rate of retinoic acid production reached saturation with increased concentration of retinal. When WIN 18446 inhibits ALDH1A2 enzyme, retinaldehyde accumulates, which might compensate for the loss of ALDH1A2 enzyme activity and promote retinoic acid synthesis to saturation^[4]. By adding dithiothreitol (DTT) to retain ALDH1A2 enzyme activity, ALDH1A2 enzyme inhibition assay still could not be reversed, indicating that WIN 18446 might inhibited ALDH1A2 enzyme directly^[4].

In vivo, C57BL/6 male mice were fed WIN 18446 (2mg/g diet) or AIN9M (control diet) for 3, 7, 14, 21, and 28 days^[6]. Testes weights with WIN 18446 treatment (2mg/g diet) were significantly reduced ^[6]. When further treated WIN 18446 groups with AIN9M control diet only for recovery assay after 14 days, testes weights remained smaller compared to control groups^[3,6]. After 28 days treatment of WIN 18446 (2mg/g diet), most testicular tubules exhibited severe epithelial disruption, characterized by the loss of germ cells and the presence of frequent multinucleated cells^[3,6].

References:

- [1] Amory JK, Muller CH, Shimshoni JA, Isoherranen N, Paik J, Moreb JS, Amory Sr DW, Evanoff R, Goldstein AS, Griswold MD. Suppression of spermatogenesis by bisdichloroacetyldiamines is mediated by inhibition of testicular retinoic acid biosynthesis. Journal of andrology. 2011 Jan 2;32(1):111-9.
- [2] Gray A. Overcoming the challenges in developing male contraceptives. The Pharmaceutical Journal. 2016;296:7890.
- [3] Das BC, Thapa P, Karki R, Das S, Mahapatra S, Liu TC, Torregroza I, Wallace DP, Kambhampati S, Van Veldhuizen P, Verma A. Retinoic acid signaling pathways in development and diseases. Bioorganic & medicinal chemistry. 2014 Jan 15;22(2):673-83.
- [4] Paik J, Haenisch M, Muller CH, Goldstein AS, Arnold S, Isoherranen N, Brabb T, Treuting PM, Amory JK. Inhibition of retinoic acid biosynthesis by the bisdichloroacetyldiamine WIN 18,446 markedly suppresses spermatogenesis and alters retinoid metabolism in mice. Journal of Biological Chemistry. 2014 May 23;289(21):15104-17.
- [5] Hartmann S, Froescheis O, Ringenbach F, Wyss R, Bucheli F, Bischof S, Bausch J, Wiegand UW. Determination of retinol and retinyl esters in human plasma by high-performance liquid chromatography with automated column switching and ultraviolet detection. Journal of Chromatography B: Biomedical Sciences and Applications. 2001 Feb 25;751(2):265-75.
- [6] Paik J, Snyder JM, Kim A, Haenisch M, Fogassy K, Amory JK. Kinetics of the inhibition and

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