产品名称：**AGI-5198**
产品别名：**AGI-5198**

## 生物活性：

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| **Description** | AGI-5198 is a potent and selective mutant **IDH1**$^{R132H}$ inhibitor with an **IC**$_{50}$ of 0.07 μM. |
| **In Vitro** | Measurements of R-2HG concentrations in pellets of TS603 glioma cells demonstrates dose-dependent inhibition of the mutant IDH1 enzyme by AGI-5198. AGI-5198 does not impair colony formation of two patient-derived glioma lines that express only the wild-type IDH1 allele (TS676 and TS516)[1]. Cancer cells heterozygous for the IDH1(R132H) mutation exhibits less IDH-mediated production of NADPH, such that after exposure to ionizing radiation (IR), there are higher levels of reactive oxygen species, DNA double-strand breaks, and cell death compared with IDH1 wild-type cells. These effects are reversed by the IDH1(R132H) inhibitor AGI-5198[2]. |
| **In Vivo** | AGI-5198 (450 mg/kg, p.o.) causes 50 to 60% growth inhibition of the tumor growth from human glioma xenografts. Tumors from AGI-5198- treated mice show reduced staining with an antibody against the Ki-67 protein. AGI-5198 does not affect the growth of IDH1 wild-type glioma xenografts[1]. |

**Solvent&Solubility**

***In Vitro:***

**DMSO : 7.14 mg/mL (15.44 mM;** Need ultrasonic)

| Preparing Stock Solutions | Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|---|
| | 1 mM | 2.1619 mL | 10.8094 mL | 21.6188 mL |
| | 5 mM | 0.4324 mL | 2.1619 mL | 4.3238 mL |
| | 10 mM | 0.2162 mL | 1.0809 mL | 2.1619 mL |

*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。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

***In Vivo:***

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：

——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

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 0.71 mg/mL (1.53 mM); Clear solution

此方案可获得 ≥ 0.71 mg/mL (1.53 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 7.1 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

2.请依序添加每种溶剂： 10% DMSO →90% corn oil

Solubility: ≥ 0.71 mg/mL (1.53 mM); Clear solution

此方案可获得 ≥ 0.71 mg/mL (1.53 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。

以 1 mL 工作液为例，取 100 μL 7.1 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。

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| **References** | [1]. Rohle D, et al. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science. 2013 May 3;340(6132):626-30.<br>[2]. Molenaar RJ, et al. Radioprotection of IDH1-Mutated Cancer Cells by the IDH1-Mutant Inhibitor AGI-5198. Cancer Res. 2015 Nov 15;75(22):4790-802. |

**实验参考：**

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| **Cell Assay** | TS603 cells are grown in medium containing either AGI-5198 (1.5μM) or DMSO vehicle control. One week prior to harvest cells are ransferred to differentiation medium (DMEM F12; 15 mM HEPES; 0.06% glucose; B27 without vitamin A; N2; Insulin/transferrin; 1% FBS) containing freshly added retinoic acid (1μM). ChIP of non-crosslinked cells is then carried out using established ChIP methods. 350 μg of lysate is immunoprecipitated-using anti-H3K9Me3, H3K27me3 or Rabbit Control IgG. After washing, ChIP DNA is eluted from protein G beads and analyzed by RT-PCR using SYBR green. Relative occupancy is calculated using the standard curve method and fold enrichment versus IgG. Enrichment in AGI- 5198-treated cells is normalized to vehicle control. Means and standard deviation are calculated from 4 technical replicates. [1] |
| **Animal Administration** | SCID mice are injected subcutaneously with $10^6$ glioma cells, which are suspended in 100 μL of a 50:50 mixture of growth media and Matrigel. Once tumors have reached a measurable size, mice are randomized into the indicated treatment groups. [1] |
| **Kinase Assay** | Inhibitory potency against the IDH2 R140Q and IDH2 R172K enzymes is determined in an endpoint assay in which the amount of NADPH remaining at the end of the reaction is measured by the addition of a large excess of diaphorase and resazurin. IDH2 R140Q is diluted to 0.25 μg/mL in 40 μL 1X Assay Buffer (150 mM NaCl, 50 mM potassium phosphate pH 7.5, 10 mM $MgCl_2$, 10% glycerol, 2 mM B-ME, 0.03% BSA) and incubated for 16 hours at 25°C in the presence of 1 μL of compound in DMSO. The reaction is started with the addition of 10 μL of Substrate Mix (20 μM NADPH, 8 μM alpha-ketoglutarate, in 1X Assay Buffer) and incubated for 1 hour at 25°C. Then, remaining NADPH is measured by the addition of 25 μL of Detection Mix (36 μg/mL diaphorase, 18 μM resazurin in 1X Assay buffer), incubated for 5 minutes at 25°C, and read as described above. IDH2 R172K is assayed as for IDH2 R140Q with the following modifications: 1.25 μg/mL of protein is used, the Substrate Mix contained 50 μM NADPH and 6.4 μM alpha-ketoglutarate, and the compound is incubated for 1 hour before starting the reaction. [1] |
| **References** | [1]. Rohle D, et al. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science. 2013 May 3;340(6132):626-30.<br>[2]. Molenaar RJ, et al. Radioprotection of IDH1-Mutated Cancer Cells by the IDH1-Mutant Inhibitor AGI-5198. Cancer Res. 2015 Nov 15;75(22):4790-802. |