产品名称：**AZD2858**
产品别名：**AZD2858**

生物活性：

Description	AZD2858 is a potent, orally active GSK-3 inhibitor, with IC_{50}s of 0.9 and 5 nM for GSK-3α and GSK-3β, respectively, used in the research of fracture healing.

IC_{50} & Target [4]

GSK-3α	GSK-3β	CDK5/p25	Haspin	CDK5/p35
0.9 nM (IC_{50})	5 nM (IC_{50})	356 nM (IC_{50})	366 nM (IC_{50})	387 nM (IC_{50})
DYRK2	CDK2/cyclin A	CDK1/cyclin B	PIM3	TLK2
491 nM (IC_{50})	810 nM (IC_{50})	1246 nM (IC_{50})	1269 nM (IC_{50})	1381 nM (IC_{50})
PKD2	CDK2/cyclin E	Aurora-A		
2462 nM (IC_{50})	3310 nM (IC_{50})	4966 nM (IC_{50})		

In Vitro	AZD2858 (1 μM) increases β-catenin levels after a short period of time in human osteoblast cells. AZD2858 inhibits GSK-3β dependent phosphorylation with an IC_{50} of 68 nM. AZD2858 (10 nM) has no effect on β-catenin levels[1]. AZD2858 increases TAZ expression and osterix expression both by 1.4-fold, with EC_{50} of 440 nM and 1.2 μM, respectively, in hADSC. AZD2858 also induces a marked increase in osteogenic mineralisation in hADSC[3]. AZD2858 (AR28) demonstrates from 70- to greater than 6000-fold selectivity over a panel of other kinases and an IC_{50} of 5 nM. AR28 inhibits GSK-3 in murine cells and indicates activation of the canonical Wnt/β-catenin signaling cascade. AR28 (50, 10, and 1 nM) enhances the clonogenic ability of mesenchymal progenitors with osteogenic and adipogenic potential. AR28 (50 μM) also enhances the differentiation ability of mesenchymal progenitors to the osteogenic but not adipogenic lineage in vitro[4].
In Vivo	AZD2858 (20 mg/kg) causes a dose-dependent increase in trabecular bone mass compared to control after a two-week treatment with a maximum effect[1]. AZD2858 exhibits a substantial effect on fracture healing. AZD2858 (20 mg/kg) causes an increase in cortical BMC of 9%, cortical area of 10%, and cortical thickness of 11% at 3 weeks in the non-operated right femur of rats[2]. AZD2858 (30 μmol/kg/day) alters the biomarkers of bone turnover with statistically significant increases in P1NP and decreases in TRAcP-5b seen from 3 days of treatment and onwards. AZD2858 demonstrates significant changes in serum bone turnover markers (P1NP and TRAcP-5b) and femur bone formation after only 7 days of daily dosing[3]. AZD2858 (AR28, 30 mg/kg, s.c.) stimulates an increase in an initial wave of mesenchymal progenitors with osteogenic and adipogenic potential and drives their differentiation to the osteogenic lineage in BALB/c mice. AR28 (30 mg/kg, s.c.) enhances the proliferation of committed hematopoietic progenitors and their differentiation to the osteoclast lineage but does not prevent an overall increase in bone mass[4].

In Vitro:

DMSO : 12.5 mg/mL (27.56 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.2050 mL	11.0249 mL	22.0497 mL
5 mM	0.4410 mL	2.2050 mL	4.4099 mL
10 mM	0.2205 mL	1.1025 mL	2.2050 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。

Solvent&Solubility	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: 1.25 mg/mL (2.76 mM); Suspended solution; Need ultrasonic 此方案可获得 1.25 mg/mL (2.76 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 12.5 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% (20% SBE-β-CD in saline) Solubility: ≥ 1.25 mg/mL (2.76 mM); Clear solution 此方案可获得 ≥ 1.25 mg/mL (2.76 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 12.5 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。
References	[1]. Marsell R, et al. GSK-3 inhibition by an orally active small molecule increases bone mass in rats. Bone. 2012 Mar;50(3):619-27. [2]. Sisask G, et al. Rats treated with AZD2858, a GSK3 inhibitor, heal fractures rapidly without endochondral bone formation. Bone. 2013 May;54(1):126-32. [3]. Gilmour PS, et al. Human stem cell osteoblastogenesis mediated by novel glycogen synthase kinase 3 inhibitors induces bone formation and a unique bone turnover biomarker profile in rats. Toxicol Appl Pharmacol. 2013 Oct 15;272(2):399-407. [4]. Gambardella A, et al. Glycogen synthase kinase-3α/β inhibition promotes in vivo amplification of endogenous mesenchymal progenitors with osteogenic and adipogenic potential and their differentiation to the osteogenic lineage. J Bone Miner Res. 2011 Apr;26(4):811-21.

实验参考：

Animal Administration	Each rat is dosed orally with vehicle or AZD2858 using a plastic gavage tube. The dose volume is 10 mL/kg. The vehicle consists of deionized water adjusted to pH 3.5±0.1. Formulations are adjusted to pH 3.5±0.1. The doses are 0, 0.2, 2 or 20 mg/kg respectively and administered either twice daily (TD), once daily (OD), every other day (O/2D), or every fourth day (O/4D) for 14 days. In total, the protocol results in 13 groups with 8 animals in each group (104 animals). At 7 days after the start of the study, and again 2 days prior to the scheduled terminal necropsy, each animal is injected subcutaneously with a bicarbonate buffered calcein solution (8 mg/kg, 1 mL/kg)[1].
	The potency of compounds at GSK-3β and cyclin-dependent protein kinase 2 (CDK2, kinase with closest homology to GSK-3β) is assessed using Z-LYTE™ Kinase assay kit in the presence of 7 and 80 μM ATP respectively. A ratiometric method is used to calculate the ratio of donor emission (445 nm) to acceptor emission (520 nm) after excitation of the donor fluorophore at 400 nm to quantitate the reaction progress. Kinase selectivity with AR79, AZD2858 and AZ13282107 are determined

Kinase Assay	using the KinaseProfiler Service or University of Dundee Kinase. Over 80 different kinases are assessed at a single concentration of 1 or 10 μM of AR79, AZD2858 and AZ13282107. Concentration-inhibition 10-point curves to compounds that show activity are constructed to determine pIC_{50} estimations. Also, in some kinase assays these pIC_{50} estimations are converted to binding affinity values (pKi) using the Cheng-Prussoff equation to correct for the concentration of ATP used[3].
References	[1]. Marsell R, et al. GSK-3 inhibition by an orally active small molecule increases bone mass in rats. Bone. 2012 Mar;50(3):619-27. [2]. Sisask G, et al. Rats treated with AZD2858, a GSK3 inhibitor, heal fractures rapidly without endochondral bone formation. Bone. 2013 May;54(1):126-32. [3]. Gilmour PS, et al. Human stem cell osteoblastogenesis mediated by novel glycogen synthase kinase 3 inhibitors induces bone formation and a unique bone turnover biomarker profile in rats. Toxicol Appl Pharmacol. 2013 Oct 15;272(2):399-407. [4]. Gambardella A, et al. Glycogen synthase kinase-3α/β inhibition promotes in vivo amplification of endogenous mesenchymal progenitors with osteogenic and adipogenic potential and their differentiation to the osteogenic lineage. J Bone Miner Res. 2011 Apr;26(4):811-21.