

Product Data Sheet

Product Name: TBB
Cat. No.: GC12181

Chemical Properties

Cas. No. 17374-26-4

Chemical Name 4,5,6,7-tetrabromo-2H-benzotriazole

SMILES C1(=C(C2=NNN=C2C(=C1Br)Br)Br)Br

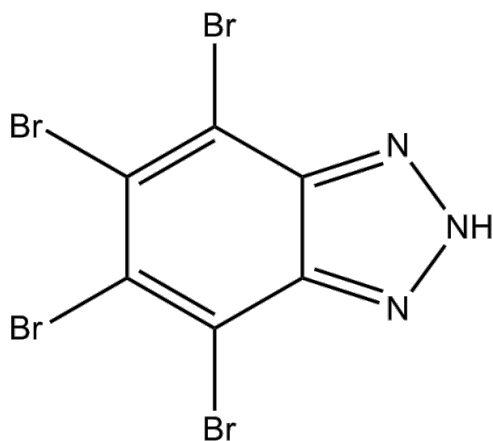
Formula $C_6HBr_4N_3$ M.Wt 434.71

Solubility ≥ 159.2 mg/mL in DMSO, ≥ 10.42 mg/mL in EtOH with ultrasonic
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

Cell experiment [1]:

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Cell lines

PC-3 cells

Preparation Method

PC-3 cells were cultured routinely in RPMI-1640 media, respectively, which were supplemented with 10% FBS, penicillin (100U/ml) and streptomycin (100µg/ ml) at 37°C in a humidified atmosphere of 5% CO₂. Cells were seeded at 5x10⁴ cells/well in 24-wells plates and cultured for 72h. TBB (final concentration 60µM), Camptothecin (CPT) (final concentration 5.8nM), 2-deoxyglucose (2-DG; final concentration 0.5mM) or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (final concentration 13.3ng/ml) were added to the medium individually or in a combination for 24h. Then the medium with the agent was removed and the cells were cultured for another 24h in normal medium. After that, 500µl of MTT mixture (0.5mg/ml in medium without phenol red) was added to each well and incubated for an additional 1h at 37°C. The formazan crystals were diluted in 250µl of DMSO. The absorbance was measured at 570nm.

Reaction
Conditions

60µM; 24h

Applications

TBB reduced the viability of PC-3 cells either alone or combined with anticancer agents CPT or TRAIL when a proper time schedule of the administration is applied.

Animal experiment [2]:

Animal models

C57BL/6J mice

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Preparation Method	Proliferative retinopathy in neonatal mice was induced in heterozygous C57BL/6J mice. Mice from the retinopathy group were placed in 75% oxygen at postnatal day 7 and maintained in these conditions with their nursing mothers for 5 days. These mice were then returned to normal air and maintained for another 5 days. Nor-moxic control mice were maintained in normal air for the same duration as test mice and under the same conditions of light cycle and temperature. For in vivo administration, the CK2 inhibitors emodin and TBB were dissolved in PBS (pH 7.2) with 20% polyethylene glycol 400 (PEG 400) and 2% Tween-80. This solvent did not elicit any animal tolerance problems, unlike ethanol or DMSO. Inhibitors were injected intraperitoneally in volumes of 50µL or less per mouse at doses of 15 to 30mg/kg body weight, twice daily from day 11 to day 17. Control mice were injected with PEG-Tween vehicle alone. At the end of the experiment, mice were euthanatized by intraperitoneal injection with tribromoethanol (0.1ml/g body weight). The eyes were enucleated and fixed in 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) for 18 hours for further analysis.
Dosage form	30 to 60mg/kg/day for 6 days; i.p.
Applications	TBB reduced retinal neovascularization by approximately 60% in the mouse OIR model with minimal effects on the main vascular tree but marked suppression or elimination of neovascular tufts.

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References:

- [1] Orzechowska, E., Kozłowska, E., Staroń, K., & Trzcińska-Danielewicz, J. (2012). Time schedule-dependent effect of the CK2 inhibitor TBB on PC-3 human prostate cancer cell viability. *Oncology reports*, 27(1), 281–285.
- [2] Ljubimov, A. V., Caballero, S., Aoki, A. M., Pinna, L. A., Grant, M. B., & Castellon, R. (2004). Involvement of protein kinase CK2 in angiogenesis and retinal neovascularization. *Investigative ophthalmology & visual science*, 45(12), 4583–4591.

Background

TBB is a cell-permeable and ATP-competitive CK2 inhibitor with an IC₅₀ of 0.15μM for rat liver CK2^[1]. TBB also inhibits three other kinases with less potency: CDK2 (IC₅₀=15.6μM), phosphorylase kinase (IC₅₀=8.7μM) and glycogen synthase kinase 3β (GSK3β) (IC₅₀=11.2μM). All other kinases tested have IC₅₀ values 50-fold greater than that for CK2^[2]. TBB is commonly used for studying the role of CK2 in cell signal transduction and gene expression regulation, and can also be applied in the research of related diseases^[3-5].

In vitro, after treatment of PC-3 cells with 60μM TBB(24h) followed by a 24h period when cells were cultured without TBB, the viability was considerably lowered, and the time schedule-dependence of the effect was maintained when TBB was administered in combination with Camptothecin (CPT)^[6].

In vivo, intraperitoneal injection of TBB (60mg/kg/day for 6 days) reduced retinal neovascularization by approximately 60% in the mouse oxygen-induced retinopathy (OIR) model, with minimal effects on the main vascular tree but marked suppression or elimination of neovascular tufts^[7].

References:

- [1] Pagano, M. A., Bain, J., Kazimierczuk, Z., Sarno, S., Ruzzene, M., Di Maira, G., Elliott, M., Orzeszko, A., Cozza, G., Meggio, F., & Pinna, L. A. (2008). The selectivity of inhibitors of protein kinase CK2: an update. *The Biochemical journal*, 415(3), 353–365.
- [2] De Moliner, E., Brown, N. R., & Johnson, L. N. (2003). Alternative binding modes of an inhibitor to two different kinases. *European journal of biochemistry*, 270(15), 3174–3181.
- [3] Sarno, S., Reddy, H., Meggio, F., Ruzzene, M., Davies, S. P., Donella-Deana, A., Shugar, D., &

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Pinna, L. A. (2001). Selectivity of 4,5,6,7-tetrabromobenzotriazole, an ATP site-directed inhibitor of protein kinase CK2 ('casein kinase-2'). *FEBS letters*, 496(1), 44–48.

[4] Chen, Z., Chen, Q., Huang, J., Gong, W., Zou, Y., Zhang, L., Liu, P., & Huang, H. (2018). CK2 α promotes advanced glycation end products-induced expressions of fibronectin and intercellular adhesion molecule-1 via activating MRTF-A in glomerular mesangial cells. *Biochemical pharmacology*, 148, 41–51.

[5] Zwicker, F., Hauswald, H., Weber, K. J., Debus, J., & Huber, P. E. (2021). *In Vivo* Evaluation of Combined CK2 Inhibition and Irradiation in Human WiDr Tumours. *In vivo (Athens, Greece)*, 35(1), 111–117.

[6] Orzechowska, E., Kozłowska, E., Staroń, K., & Trzcińska-Danielewicz, J. (2012). Time schedule-dependent effect of the CK2 inhibitor TBB on PC-3 human prostate cancer cell viability. *Oncology reports*, 27(1), 281–285.

[7] Ljubimov, A. V., Caballero, S., Aoki, A. M., Pinna, L. A., Grant, M. B., & Castellon, R. (2004). Involvement of protein kinase CK2 in angiogenesis and retinal neovascularization. *Investigative ophthalmology & visual science*, 45(12), 4583–4591.

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