# A-446: A Chemical Probe for GLS

Version 1.1 (7th July 2021)

**Web link for more details: https://www.sgc-ffm.uni-frankfurt.de/#!specificprobeoverview/A-446**

## Overview

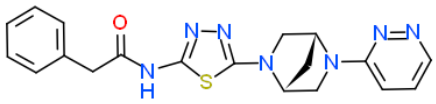
The aminohydrolase GLS is responsible for the conversion of glutamine to glutamate. There are multiple glutaminase isoforms in humans. The splice variant GAC of GLS, the kidney isoform, is overexpressed in many cancer cell lines. GLS inhibition has been targeted as a potential oncology therapy based on the hypothesis that inhibition can selectively starve cancer cells.

## Summary

| | |
|---|---|
| Chemical Probe Name | A-446 |
| Negative control compound | A-426 |
| Target(s) (synonyms) | GLS (Glutaminase, GLS1) |
| Recommended *in vitro* assay concentration | Use at concentration of 10 nM for A-446 and A-426; use with control for best interpretation of data |
| Suitability for *in* vivo use and recommended dose | Tested in mice with 50 - 200 mg/kg BID. A-446 showed moderate dose dependent tumor growth inhibition across multiple lung cancer cell lines in mouse xenograft studies. |
| Publications | None at time of writing |
| Orthogonal chemical probes | |
| *In vitro* assay(s) used to characterise | GAC enzyme activity assay |
| Cellular assay(s) for target-engagement | CTG assay in A549 cells |

## Chemical Probe & Negative Control Structures and Use

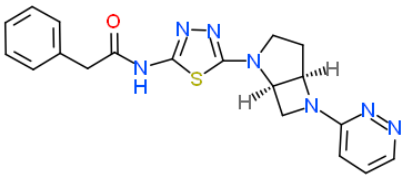
A-446 Chemical Probe



**SMILES**: CC[C@@H]1CN(CCN1c1cccnn1)c1nnc(NC(Cc2ccccc2)=O)s1

**InChiKey:** YZQFDCMJURBEKW-MRXNPFEDSA-N
**Molecular weight:** 409.17
**Storage**: As a dry powder or as DMSO stock solutions (10 mM) at -20 °C. DMSO stocks beyond 3-6 months or 2 freeze/thaw cycles should be tested for activity before use
**Dissolution**: Soluble in DMSO up to 10 mM; use only 1 freeze/thaw cycle per aliquot

A-426 Negative Control



**SMILES**:
[H][C@@]12CCN(c3nnc(NC(Cc4ccccc4)=O)s3)[C@]1([H])CN2c1cccnn1
**InChiKey**: VCDTXROFDBHEIP-HUUCEWRRSA-N
**Molecular weight:** 393.14
**Storage**: As a dry powder or as DMSO stock solutions (10 mM) at -20 °C. DMSO stocks beyond 3-6 months or 2 freeze/thaw cycles should be tested for activity before use
**Dissolution**: Soluble in DMSO up to 10 mM; use only 1 freeze/thaw cycle per aliquot

## Chemical Probe Profile

*In vitro* Potency & Selectivity:

A-446 shows potent activity in the GAC (GLS) enzyme activity assay ($IC_{50}$ = 31 nM). It is selective within the target family with >1000-fold selectivity versus GLS2 ($IC_{50}$ > 30 µM; GLS2 enzyme activity assay). Closest off-targets in the CEREP binding assay at 10 µM [% inhibition of control specific binding] are ADORA3 (agonist radioligand; 76.6 %), CHRM1 (antagonist radioligand; 92.6 %) and CHRM2 (antagonist radioligand; 71.5 %). The control A-426 is > 100-fold less active in the GLS enzyme activity assay ($IC_{50}$ > 30 µM) and GLS2 enzyme activity assay ($IC_{50}$ > 30 µM).

Potency in Cells and Cellular Target Engagement:

A-446 is potent in the CTG assay in A549 cells with $IC_{50}$ = 11 nM.