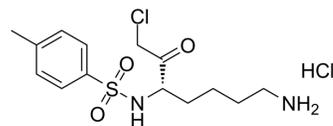


## N-alpha-Tosyl-L-lysine chloromethyl ketone hydrochloride

Cat. No.:	HY-112716
CAS No.:	4272-74-6
Molecular Formula:	C <sub>14</sub> H <sub>22</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub> S
Molecular Weight:	369.31
Target:	Others
Pathway:	Others
Storage:	-20°C, sealed storage, away from moisture * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 250 mg/mL (676.94 mM; Need ultrasonic)						
	H <sub>2</sub> O : 100 mg/mL (270.78 mM; Need ultrasonic)						
	Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg	
				1 mM	2.7078 mL	13.5388 mL	27.0775 mL
				5 mM	0.5416 mL	2.7078 mL	5.4155 mL
10 mM				0.2708 mL	1.3539 mL	2.7078 mL	
Please refer to the solubility information to select the appropriate solvent.							
In Vivo	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.08 mg/mL (5.63 mM); Clear solution						
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (5.63 mM); Clear solution						
	3. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.08 mg/mL (5.63 mM); Clear solution						

### BIOLOGICAL ACTIVITY

Description	N-alpha-Tosyl-L-lysine chloromethyl ketone (TLCK), a trypsin like protease inhibitor, sensitizes HeLa cells to Fas-mediated cell death.
In Vitro	N-alpha-Tosyl-L-lysine chloromethyl ketone exhibits an inhibitory effect on IFN-γ activities. The effect of TLCK is studied on the IFN-γ sensitization of HeLa cells towards cell death mediated by anti-Fas. Lower concentration of anti-Fas (10 ng/mL) are used to examine the interaction among the three effectors simultaneously, that is, anti-Fas, TLCK and IFN-γ. TLCK by itself up to 50 μM concentration exhibits a small decrease in cell viability. Beyond 50 μM, a dose dependent decrease in cell viability is observed. IFN-γ slightly reduces cell viability on its own. Addition of anti-Fas (10 ng/mL) results in a slight

decrease in cell survival, which is enhanced more than additively in the presence of TLCK, most prominently between 50 and 100  $\mu\text{M}$ . Upon addition of both anti-Fas and IFN- $\gamma$ , a decrease ( $\approx 46\%$ ) in cell viability is observed. Moreover, the decrease in cell survival is further enhanced upon addition of higher concentrations of TLCK, 25  $\mu\text{M}$  and more<sup>[1]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## PROTOCOL

### Cell Assay <sup>[1]</sup>

HeLa cell line (human cervical cancer cells) is cultured in DMEM supplemented with 10% fetal bovine serum (FBS), L-glutamine (300 mg/L), penicillin (100 U/ml) and streptomycin (100  $\mu\text{g}/\text{ml}$ ) at 37°C in 5% CO<sub>2</sub>. HT-29 cell line (human colorectal adenocarcinoma) is cultured in RPMI supplemented with 10% fetal bovine serum (FBS), L-glutamine (300 mg/L), penicillin (100 U/mL) and streptomycin (100  $\mu\text{g}/\text{mL}$ ) at 37°C in 5% CO<sub>2</sub>. The cells are split every second day to keep the cell growth in logarithmic phase. The cells are routinely tested for mycoplasma. The cells are treated with different concentrations of TPCK or TLCK (5, 10, 25, 50, 100, 150, and 200  $\mu\text{M}$ ) for 30 min and/or with different concentrations of IFN- $\gamma$  for 2 h followed by treatment with different concentrations of anti-Fas for 2 or 4 days for each specific experiment. The control cells are treated with the respective vehicle only. Cell viability analysis of HeLa and HT-29 cells is assessed by their XTT reduction activity. 100  $\mu\text{L}$  of  $2 \times 10^4$  cells/mL is incubated with treatments at the indicated time. At the end of the incubation period, 25  $\mu\text{L}$  of 1 mg/mL XTT solution (containing 0.2 mM phenazine methosulphate (PMS) is added and the cells are incubated for an additional 1 h. The OD values are measured using an ELISA reader at 450 nm with a reference wavelength of 650 nm<sup>[1]</sup>.

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## CUSTOMER VALIDATION

- Cell Death Dis. 2021 Jan 7;12(1):42.

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## REFERENCES

[1]. Shadrin N, et al. Serine protease inhibitors interact with IFN- $\gamma$  through up-regulation of FasR; a novel therapeutic strategy against cancer. Exp Cell Res. 2015 Jan 15;330(2):233-9.

**Caution: Product has not been fully validated for medical applications. For research use only.**

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