N-TOSYL-L-PHENYLALANINE CHLOROMETHYL KETONE
Sigma Prod. No. T4376

CAS Number: 402-71-1
SYNONYMS: TPCK;
Tosylphenylalanylchloromethane; L-
Tolylsulfonylphenylalanyl Chloromethyl Ketone; N-
Tosyl-L-Phenylalaninechloromethane; L-1-
Tosylamido-2-Phenylethyl Chloromethyl Ketone; L-
N-(α-(Chloroacetyl)Phenethyl)-p-
Toluenesulfonamide; Tos-Phe-CH2Cl

PHYSICAL DESCRIPTION:
Appearance: white powder
Melting Point: 106-108°C
Molecular Formula: C17H18ClNO3S
Molecular Weight: 351.8

METHOD OF PREPARATION:
TPCK is synthetically prepared. A method of preparation has been reported.

STABILITY / STORAGE AS SUPPLIED:
TPCK is expected to be stable for at least two years when stored desiccated at -20°C.

SOLUBILITY / SOLUTION STABILITY:
Stock solutions of 10 mM can be prepared in methanol or ethanol and are stable for several months at 4°C. TPCK is soluble in DMSO; preparation of 10 mg/ml solution was described. The effective concentrations in aqueous solutions are in the range of approximately 10-100 µM. Working solutions are stable only for several hours.

USAGE / APPLICATIONS:
TPCK irreversibly inhibits the serine protease α-chymotrypsin. TPCK has been shown to specifically alkylate the histidine-57 moiety in the active center of chymotrypsin and chymotrypsin-like serine proteases. The nature of the enzyme-inhibitor complex and the mechanism of inactivation have been reported.
TPCK inhibits the active enzyme and not the zymogen precursor nor enzyme-inhibitor complex. Trypsin (not inactivated) can be treated with TPCK for removal of chymotrypsin activity. TPCK also inactivates some cysteine proteases such as bromelain, ficin and papain by reacting with the active sulfhydryl group of the enzyme rather than on the imidazole group of a histidyl residue, as in the case of chymotrypsin. TPCK inhibited the catalytic subunit of cAMP-dependent protein kinase in both rat and rabbit muscle and protein kinase C (IC$_{50}$=8mM) probably by the alkylation of a sulfhydryl-containing amino acid residue in the enzyme active center. TPCK (IC$_{50}$, 5 µM) inhibited the mitogen-induced activation of pp70, a mitogen-regulated serine/threonine kinase involved in the G$_1$ to S phase transition of the cell cycle.

TPCK (25 µM) reportedly induced early morphological and biochemical changes associated with apoptosis, inhibited internucleosomal cleavage in rat thymocytes, and (at 10 µM) in human promyelocytic leukemic cell line (HL-60) and affected other apoptotic events induced by camptothecin (CAM). TPCK (30 µM) induced tyrosine phosphorylation of a substrate (molecular weight, 42,000) in HL-60 cells and in human monocytes in conjunction with inhibition of apoptosis-associated CAM-induced internucleosomal DNA fragmentation. The reported results suggest a link between protein phosphorylation (as a signalling event) and regulation of apoptosis. TPCK reacted with the Rb-binding core of human papillomavirus HPV-18 E7 oncoprotein and destroyed its Rb-binding ability. TPCK (5 x 10$^{-4}$ M) was shown to be a selective irreversible inhibitor of the complex of S$_1$S$_3$-factors in the cell-free protein-synthesizing system from B. stearothermophilus. TPCK (25 µM) completely inhibited induction of NF-kB (transcriptional activator protein) activity as well as the decay of the subunit IkB (necessary for activation) in response to phorbol 12-myristate 13-acetate (PMA) in cells. TPCK (mM concentration) inhibited E. coli proteases, Re (1 mM); Fa (80% at 0.5 mM) and So (1 mM). TPCK weakly inhibited chymase (I$_{50}$=240 µM), a serine protease, in rat peritoneal mast cells.

In vitro nitric oxide production from immunostimulated alveolar macrophages of the mice and rat was inhibited by TPCK (3x10$^{-7}$-3x10$^{-4}$M). TPCK interfered with the lipopolysaccharide induced nitric oxide synthase gene expression in rat alveolar macrophages. TPCK (100-200 µg/ml) inhibited the growth of simian virus 40-transformed and untransformed 3T3 cells probably by inhibition of cellular protein synthesis. Addition of 20-30 µg/ml of TPCK to HeLa cells, virus-transformed 3T3 mouse fibroblasts and mouse plasmacytoma culture cells irreversibly inhibited initiation of protein synthesis. TPCK irreversibly inhibited the interaction between the Elongation Factor Tu and phenylalanyl transfer RNA and prevented its transfer to the ribosome for formation of polyphenylalanine. TPCK (5.7 µM) increased arachidonic acid metabolism (prostacyclin production increased) in rat liver cells stimulated by agonist agents.
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USAGE / APPLICATIONS: (continued)

TPCK (284 µM) inhibited the activity of purified CMP-sialic acid: lactosylceramide α(263) sialyltransferase (GM3 synthase, a GM3 ganglioside-forming enzyme) from rat liver.37 TPCK (1 mM) inhibited about 78% of a chymotrypsin-like activity of human leucocyte granules.38 To prevent proteolytic degradation throughout isolation of proteins, 1 mM each of TPCK and trypsin inhibitor TLCK (L-tosyl-lysine chloromethyl ketone) was used in the isolation of histones from chicken erythrocytes.39

GENERAL NOTES:

TPCK is an irreversible inhibitor of chymotrypsin, of chymotrypsin-like serine proteases, and of some cysteine proteases.3,16,18,40,41 TPCK which is hydrophobic and of relatively low molecular weight is likely to penetrate the plasma membrane and act within the cell, i.e., affecting cell apoptotic events.7,25

REFERENCES:

1. Chemical Abstracts Registry data, American Chemical Society.
4. Sigma Quality Control data.
5. Supplier data
REFERENCES: (continued)