

Tubulins from different tissue vary in the relative abundance of specific isoforms and the nature of post-translational modifications. These tissue type specific variants of tubulin have different biological and biochemical properties. It follows that the development of anti-tubulin ligands would benefit from the use of tubulin species purified from tissues that are relevant to the pathology under investigation. The specificity of ligands for a particular tubulin variant can be determined by performing comparative studies with both plant and neuronal tubulins. We have advanced this concept by developing the Tubulin Ligand Index (TLI) system. In this system, IC50 values for inhibitory compounds or EC50

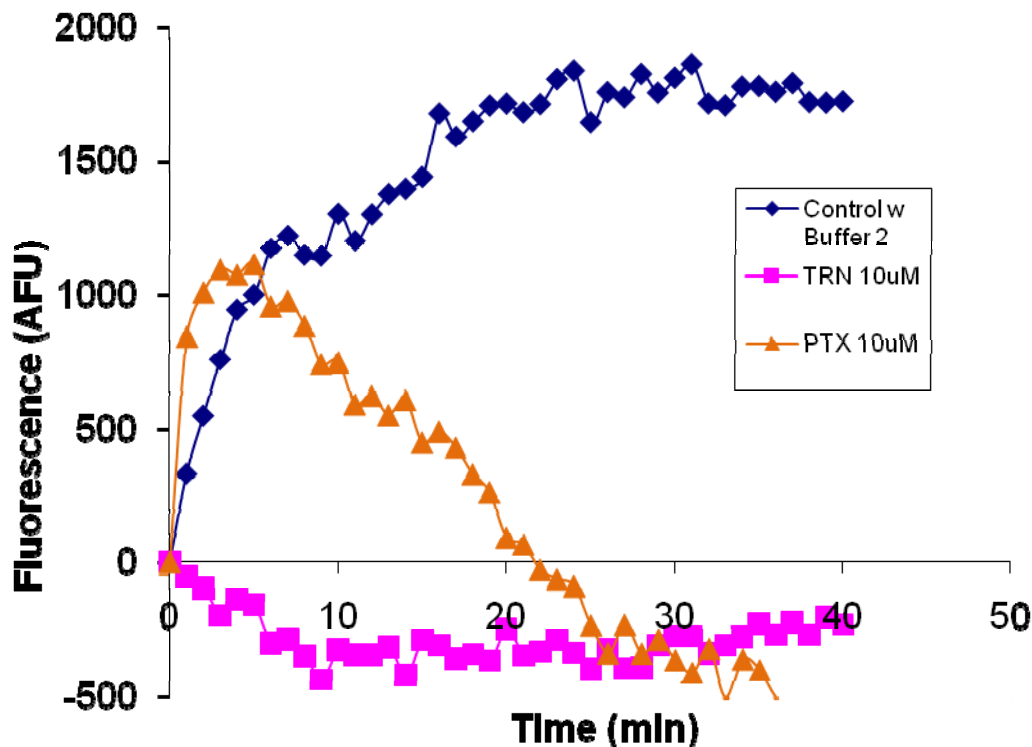
values for stabilizing molecules are determined in polymerization assays using plant and neuronal tubulins. The IC50 or EC50 values for each tubulin variant are analyzed as a ratio (neuronal/plant) and allow for determinations of the relative specificity for each tested compound. TLI values greater than 1.0 indicate that a particular compound is more active on plant tubulin. Conversely, TLI values less than 1.0 suggest that a compound is more specific for neuronal tubulin. Table 1 summarizes data from a study comparing the affects of several tubulin ligands on soybean tubulin in comparison to bovine neuronal tubulin using the TLI system.

Table 1: Comparison of IC50's of four compounds acting on neuronal and soybean tubulin polymerization.

Ligand	EC50* or IC50* Neuronal Tubulin (μM)	EC50* or IC50* Soybean Tubulin (μM)	Tubulin Ligand Index Ratio (Neuronal/Soybean)
Paclitaxel	0.48	3.60	0.133
Nocodazole	3.40	>100	<0.034
Pendimethalin	>100	3.60	>28
Trifluralin	>100	1.90	>52

* = Values based on the rate of microtubule polymerization, CV +/- 24%.

Figure 1: Example raw data from soybean tubulin undergoing polymerization.



Legend to Figure 1: Soybean tubulin was polymerized in G-PEM plus 8% DMSO containing 10 μ M Dapi as fluorescence reporter (See BK010S kit). 10 μ M Paclitaxel (PTX) and 10 μ M Trifluran (TRN) were added to separate reactions.

Citations:

Tresch S, Niggeweg R. and Grossmann K. (2008). The herbicide flamprop-M-methyl has a new antimicrotubule mechanism of action. *Pest Manag Sci* 64:1195–1203.