Structural features of the lolitrem family of fungal alkaloids important for inhibition of large conductance calcium-activated potassium (BK) channels

J.E. Dalziel,[1] W.I. Imlach,[1] J. Dunlop[1] and S.C. Finch,[2] [1]AgResearch, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North 4442, New Zealand and [2]Ruakura Research Centre, East Street, Private Bag 3123, Hamilton 3240, New Zealand.

The lolitrem family of fungal alkaloids originate *in planta* as a result of grass-endophyte symbiosis. These indole diterpene compounds are secondary metabolites that are produced when perennial ryegrass is infected with the endophytic fungus *Neotyphodium lolii*. The most abundant compound is lolitrem B which is the main compound responsible for a neurological condition called 'ryegrass staggers' which impairs motor function in grazing animals. We recently showed that motor function deficits induced by lolitrem B in mice are mediated by BK channels. It is not known where lolitrem B binds to the BK channel, nor which structural features of the inhibitor confer its high potency (IC50 = 4 nM). The aim of this study was to determine which components of the lolitrem structure are important for BK channel inhibition. We used a suite of eight lolitrem compounds, four of which were isolated from endophyte-infected ryegrass seed, from which the other four were obtained by chemical modification. Compounds with subtle structural differences were chosen, so that data on their ability to inhibit BK channels would provide insights into the key structural features required for inhibition. Using inside-out membrane patches from *hSlo* HEK cells expressing human BK channels, we examined the lolitrem concentration-response relationship and the conductance-voltage relationship. The presence of an isoprene unit or an acetate group conferred an increase in apparent affinity for BK channels, whereas stereoisomer conformation had less influence.