A COMPARISON BETWEEN COMPOUND POTENCY IN AN *IN VITRO* PHOSPHOLIPIDOSIS ASSAY AND AT THE HERG-ENCODED POTASSIUM CHANNEL

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Cationic amphiphilic drugs (CADs) are associated with phospholipidosis (PLD), an effect characterised by intracellular accumulation of phospholipids and drug in concentric lamellar bodies. In physicochemical terms, inhibitors of the human ether-a-go-go related gene (hERG) encoded potassium channel are similar to PLD-inducing compounds. In view of this, and the observation that some PLD-inducing compounds *in vivo* have been reported to prolong the QT interval (e.g. fluoxetine; Gonzalez-Rothi *et al.*, 1995.), we tested whether there was any correlation between compound potency in an *in vitro* PLD assay (EC_{50}) and at the hERG channel (IC_{50}).

Thirty-nine compounds were tested in both assays. Briefly, the potency (EC_{50}) of PLD inducing compounds was determined by measuring accumulation of a surrogate phospholipid; N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (NBD-PE) in a mouse spleen macrophage cell line (I.13-35) using a Cellomics ArrayScan™. Cell culture and compound incubations were conducted at 37°C, cell fixation, staining and measurement of fluorescence at room temperature. Cells were used between passages 18 - 50. The methods have been described in detail by Morelli *et al.* (2006). The potency (IC_{50}) of these compounds at the hERG-encoded channel was determined in CHO cells using IonWorksHT™. Cell culture was conducted at 37°C; otherwise all experiments were performed at room temperature (22°C). Cells were used between passages 10 - 30. The methods have been described in detail by Bridgland-Taylor *et al.* (2006). Physicochemical properties; cLogP and pK_a of the compounds were estimated by computational methods.

Although 33 of the compounds were active in both assays, there was no positive correlation between their IC_{50}s ($r = -0.35$). A t-test was performed on this data to assess the null hypothesis that the slope equals one. The associated probability of the test statistic (12) was < 0.0001 and therefore strongly rejects the null hypothesis. The cLogP and pK_a range of these compounds was 2.0-8.9 and 6.1-11.2, respectively. The 6 other compounds (cLogP and pK_a range 0.7-5.0 and 3.4-9.7, respectively) were inactive in the PLD assay but their hERG IC_{50}s ranged from 3-210 μM). The lack of a potency correlation indicates that hERG data cannot be used as a quantitative indicator of *in vitro* PLD potency or *vice versa*. However, potency data in both assays relative to cLogP and pK_a suggest that decreasing compound basicity and lipophilicity is likely to reduce the risk of both hERG inhibition and PLD *in vitro*.

Gonzalez-Rothi, R.J. *et al.* (1995). *Chest.* **107,** 1763 - 1765.
Morelli, J.K. *et al*. (2006). *Cell Biol and Toxicol*. **22**, 15-27.
Bridgland-Taylor, M. *et al*. (2006). *J. Pharmacol and Toxicol Meth*. **54**, 189-99.