Development of Selective Bisubstrate-Based Inhibitors against Protein Kinase C (PKC) Isozymes

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Study Design
Three PKC isozymes (PKCα, PKCβ, PKCγ) were selected for kinase activity substrate profiling using the Serine/Threonine PamChip® microarray. The increase in fluorescence was monitored kinetically for each substrate during 60 minutes (figure 1 top). The slope of each phosphorylation curve was calculated and converted into an initial velocity (Vini). This was carried out in triplicate for all three PKC iso-enzymes (figure 1 bottom). Next, affinity of two newly synthesized bisubstrate-based inhibitors were profiled against staurosporine on three PKC iso-enzymes (figure 2). In this study the Z’-factor (measure of assay quality) was typically 0.6–0.8 showing adequate assay performance.

Key Findings
Selective peptide substrates for each of the PKC iso-enzymes were identified on PamChip® array. In addition biselective and iso-enzyme selective compounds with higher affinity compared to staurosporine were identified using the PamChip® array.

“Author Quote”
We now evaluate all synthesized PKC inhibitor compounds for their activity against PKC using flow-through microarrays.

Background
Selectivity of kinase inhibitors is an important aspect of kinase inhibitor drug development especially for protein kinase C (PKC). A key problem with PKC is the existence of twelve high homology isoforms in humans which creates difficulties in designing selective PKC inhibitors. In this application note we describe a method to prepare bisubstrate-based inhibitors that target both the selective peptide-binding site and the ATP-binding site.

Conclusion
The PamGene platform provided the means to measure potency and selectivity, compared to other PKC isoforms, of novel nanomolar affinity, bisubstrate-based inhibitors for PKC theta.

References: