Identification and pharmacological characterisation of novel positive allosteric modulators (PAM) of Melanocortin 3 Receptors

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Melanocortin peptide hormones are produced in the pituitary by post-translational cleavage of the proopiomelanocortin (POMC) gene product. Melanocortin peptides exert numerous biological functions through activation of a sub-family of G_s-coupled Class A 7-transmemberane (7TM) receptors, namely, the 5 melanocortin receptors (MC_1, MC_2, MC_3, MC_4 and MC_5). Dysfunction of these peptide-receptor systems has been implicated in an equally diverse myriad of patho-physiological conditions relating to skin pigmentation, steroidogenesis, energy balance, food intake, sexual behaviour and inflammation, making these receptors attractive targets for therapeutic intervention.

MC_3 receptors are expressed on immune cells, including macrophages and mast cells, and activation leads to downstream attenuation of NF-κB function and reduced release of pro-inflammatory mediators (IL-1, IL-6 and IL-8). It has been hypothesised that pharmacological activation of MC_3, which has increased expression in conditions of stress such as inflammatory and mechanical joint disease, could attenuate pro-inflammatory responses and offer novel anti-arthritic therapeutic potential.

In comparison to 'direct' agonist activation, positive allosteric modulators of MC_3 could afford additional therapeutic advantage including improved receptor-subtype selectively, retention of physiologically-controlled spatial and temporal resolution as well as a self-limiting saturablility of effect. To this end we have recently configured a high-throughput screen to simultaneously identify novel agonists and PAMs of the MC_3 receptor using a functional HTRF cAMP detection assay (Cisbio), the MRCT 100K compound collection and CHO cells stably expressing MC_3 (Invitrogen).

In addition to acceptable assay performance and stability, screening at a single concentration (10µM) of test compound in the presence of an EC20 of native MC3 agonist (lys-gamma3-MSH), revealed a hit rate of 0.47% using a 50% activity cut off. Secondary studies on these putative hits, for the deconvolution of agonist and PAM mechanisms, in the absence and presence of native agonist, respectively, provided two distinct populations. Full curve follow-up on exemplars from each population revealed a range of apparent potencies and efficacies (pEC50 5 - 7). Quantitative pharmacological analyses, including leftward-shift experiments, reinforced the distinction between agonist and PAM modalities and also identified MC_3 PAMs devoid of overt agonism/efficacy in this assay system. For a single PAM exemplar tested, no significant potentiation of metaproterenol-mediated responses were seen in the same CHO host cells transiently expressing beta-2 adrenergic receptors and using an identical HTRF cAMP detection readout.

Taken collectively, these data, albeit preliminary, are consistent with allosteric potentiation of MC3 which is most likely affinity-driven. Moreover, the apparent lack of affect in 'counter assays' expressing a different 7TM, but sharing common host cells and detection technology, suggest potentiation in the functional MC3 assay are receptor specific.

We will also present a suggested generalised follow-up screening strategy when targeting allosteric modulators of 7TM and GPCRs and the development of a recommended 'assay toolbox'. Ideally this would include the availability of non-transfected host cells, use of competitive antagonists, assay

systems with variable/controllable receptor expression and importantly, early assessment of cross species PAM pharmacology.