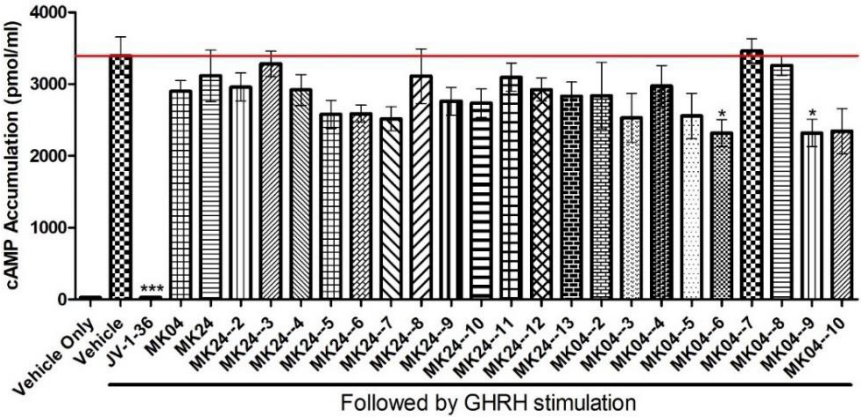
Identification and characterisation of small-molecule growth hormone-releasing hormone receptor antagonists

Radomsky, T*[1,2]; Mohamed Moosa, Z[1,3]; Anderson, R.C[1,2]; Newton, C.L[1,4]

[1] Centre for Neuroendocrinology, Faculty of Health Sciences, University of Pretoria
[2] Department of Physiology, Faculty of Health Sciences, University of Pretoria
[3] Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria
[4] Department of Immunology, Faculty of Health Sciences, University of Pretoria
*Contact details: Email: u15125239@tuks.co.za, Tel: 0798873649



Inhibition of GHRH-stimulated cAMP generation in cells expressing GHRHR by test compounds.

Introduction: In addition to their vital role in normal growth and metabolism, growth hormone releasing hormone (GHRH) and its receptor (GHRHR) act as autocrine growth factors to induce proliferation and differentiation of various cancer cell types.[1] Indeed, a GHRHR splice variant (SV1), is constitutively active and pro-proliferative and has been found to be overexpressed in many cancers, including breast cancer.[2] The GHRHR is therefore an attractive therapeutic target. Peptide antagonists that block activity of this receptor have been found to decrease cancer cell growth in vitro and in vivo,[3,4] but unfortunately, the therapeutic potential of peptide therapeutics is limited. Therefore, in this study, in silico structure-based drug discovery was utilised to identify potential small-molecule (non-peptide) GHRHR-interacting compounds and their in vitro activity, including effects on breast cancer cell growth, was determined.

Methods: The effect of the identified small-molecule compounds on GHRH-induced GHRHR signalling (and the specificity of these responses for the GHRHR), were determined by cyclic-AMP ELISA in cells expressing GHRHR (or closely related receptors). Resazurin and crystal violet assays were utilised to determine breast cancer (MCF-7) cell proliferation whilst viability in response to small-molecule compound stimulation and cytotoxicity was determined by the LDH assay.

Results: Compounds MK04-6 and MK04-9 were found to significantly decrease GHRH-induced signalling of the GHRHR (see Figure). Examination of the activity of these compounds on other closely related receptors, confirmed the specificity/selectivity of these responses for the GHRHR. MK04-6 and MK04-9 were also found to significantly decrease proliferation and viability of MCF-7 breast cancer cells exogenously expressing GHRHR, with these results not due to cytotoxicity.

Discussion and conclusion: While not as effective as a current peptide antagonist (JV-1-36) in inhibiting GHRH-induced responses, the effects of these small-molecule compounds are promising. In particular, the decrease seenin MCF-7 cell metabolism and growth due to stimulation with these compounds indicates that use of GHRHR-targeting non-peptide compounds may be a viable approach for future treatment of cell growth disorders, such as cancer. Compounds MK04-6 and MK04-9 provide a good starting point for further development/compound refinement through medicinal chemistry and in silico docking studies.

References:
1. Mayo KE, et al.. Regulation of the pituitary somatotroph cell by GHRH and its receptor. Recent Prog Horm Res. 2000;55:237–66; discussion 266-7.
2. Barabutis N, et al. Stimulation of proliferation of MCF-7 breast cancer cells by a transfected splice variant of growth hormone-releasing hormone receptor. Proc Natl Acad Sci U S A. 2007;104(13):5575–9.
3. Kahán Z, et al.. Antagonists of growth hormone-releasing hormone arrest the growth of MDA-MB-468 estrogen-independent human breast cancers in nude mice. Breast Cancer Res Treat. 2000;60(1):71–9.
4. Annunziata M, et al. The growth hormone-releasing hormone (GHRH) antagonist JV-1-36 inhibits proliferation and survival of human ectopic endometriotic stromal cells (ESCs) and the T HESC cell line. Fertil Steril. 2010 Aug;94(3):841–9.

Keywords: Growth hormone-releasing hormone receptor, non-peptide antagonists, breast cancer