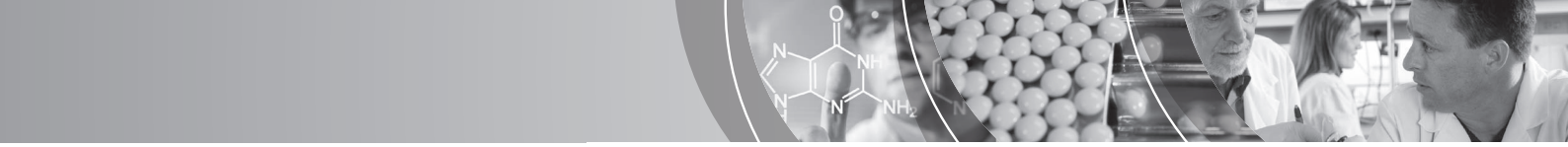

495

Role of reactive oxygen species in calcium signalling in hypoxia-induced epithelial-mesenchymal transition
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Introduction. Hypoxia is a hallmark of the cancer microenvironment and induces epithelial-mesenchymal transition (EMT) in breast cancer cells. EMT describes the transition of breast cancer cells into a more invasive phenotype. Hypoxia has been reported to cause changes in calcium (Ca^{2+}) signalling in a variety of cell types including osteosarcoma and HEK293 cells, possibly through the production of reactive oxygen species (ROS), however, this pathway has not been studied in breast cancer cells.
Aims. To investigate the role of hypoxia-mediated increases in ROS in the induction of EMT and altered Ca^{2+} signalling in MDA-MB-468 breast cancer cells.
Methods. MDA-MB-468 breast cancer cells were incubated for 24 h at 1% O_2 to induce hypoxia. ROS increases were assessed using a cell-permeable fluorogenic probe 2´,7´-dichlorodihydrofluorescin diacetate (DCFH-DA). Real time RT-PCR was used to assess the mRNA levels of 50 calcium channels and transporters in MDA-MB-468 cells in the presence and absence of hypoxia.
Results. Hypoxia increased levels of the EMT markers vimentin and N-cadherin, and increased intracellular ROS levels. Five Ca^{2+} permeable channels belonging to different classes were identified as altered by hypoxia in MDA-MB-468 breast cancer cells. Chelation of ROS with 10 mM N-acetylcysteine (NAC) significantly changed the expression pattern of these five selected Ca^{2+} channels as well as the EMT marker N-cadherin.
Discussion. These results suggest an important role of ROS in hypoxia-mediated EMT in breast cancer cells and indicate that such changes are associated with alterations in the expression of specific Ca^{2+} permeable ion channels.

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Biased agonism at the adenosine A_1 receptor: Implications for cytoprotection.
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Introduction. Adenosine A_1 receptor (A_1AR) stimulation is protective in a number of cardiovascular and neuronal conditions, however, current therapeutic targeting is limited due to bradycardia (Jacobson & Gao, 2006). Recently, the novel A_1AR agonists, VCP746 and capadenoson, have been shown to retain cytoprotective signaling in the absence of bradycardia, a phenomenon suggestive of ligand bias (Kenakin et al., 2012; Sabbah et al., 2013).
Aims. To compare the bias profile of prototypical A_1AR agonists with that of the atypical A_1AR agonists, VCP746 and capadenoson.
Methods. A propidium iodide-based assay assessed A_1AR agonist-mediated cytoprotection of CHO cells stably expressing the human A_1AR (CHO-A_1) after 24-hour serum starvation. Phosphorylation of ERK1/2 and AKT, calcium mobilization and cAMP accumulation were determined using fluorescence approaches. Functional selectivity was quantified as described previously (Kenakin et al., 2012).
Results. Functional assays demonstrated that each A_1AR agonist mediated a robust increase in ERK1/2 and AKT1/2/3 phosphorylation, calcium mobilization and inhibition of cAMP accumulation (n=3-6). In contrast to the prototypical A_1AR agonists, VCP746 and capadenoson were biased away from calcium mobilization ($p<0.05$, one-way ANOVA, Tukey Post Hoc test).
Discussion. The differential modulation of intracellular A_1AR signaling exhibited by VCP746 and capadenoson suggests that they behave in a manner that is unique to prototypical A_1AR agonists, and that this in turn may underlie their ability to mediate cytoprotection in the absence of bradycardia.

Jacobson K & Gao K (2006) Nat Rev Drug Discov 5:247-264
Kenakin T et al (2012) ACS Chem Neurosci 3:193-203
Sabbah H et al (2013) Circ Heart Fail 6:563-571

497

Novel benzamide derivatives as potent P2X7 receptor antagonists.

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Introduction. The P2X7 receptor is an ATP ligand-gated ion channel found predominantly on immune cells. It has the rare ability to form pores upon prolonged activation. Recent work has implicated the P2X7 in a number of health concerns, such as depression, neuropathic pain and rheumatoid arthritis, making it an important therapeutic drug target (1).
Aims. A range of P2X7 receptor benzamide derivatives have been synthesised, varying in size and charge, with the aim to examine their potency compared to an adamantane lead compound. It was anticipated that these new polycyclic benzamides would highlight the structural properties underlying potent P2X7 antagonism, specifically functionality and tolerability.
Methods. LPS/IFNγ-differentiated THP-1 monocytes were used to examine cell viability, as assessed by the Cell-Titer Blue assay, and dye uptake using Yo-Pro fluorescent dye was employed to assess pore formation.
Results. Of the compounds tested, the largest polycyclic motif (closo-carborane) was as potent as the lead adamantane, with a pIC_{50} (±SD) of 8.07±0.19, compared to 7.98±0.15. Interestingly, the smallest of polycycles (cubane) displayed the lowest potency (6.36±0.12). Giving the largest polycycle an anionic charge (nido-carborane) lowered its pIC_{50} to 6.43±0.1. None of the tested compounds had any effect on cell viability.
Discussion. Compounds that contained a large polycyclic cage (i.e. closo-carborane and adamantane) gave potent pIC_{50} values. However, when the closo-carborane compound was deboronated to give the anionic nido-carborane, the potency decreased substantially, suggesting that the hydrophobic pocket in the P2X7 receptor binding site does not tolerate this anionic charge. This work provides insight into the structure-activity relationships of P2X7 receptor anatagonists, with the promise of new, more potent, compounds being developed in the near future for potential therapeutic use.
(1) Skaper SD et al (2010) FASEB J 24:337-345.

499

Trapped in the act: The α4-α4 interface is an additional binding site for antagonists that can differentiate between stoichiometries of α4β2 nAChRs

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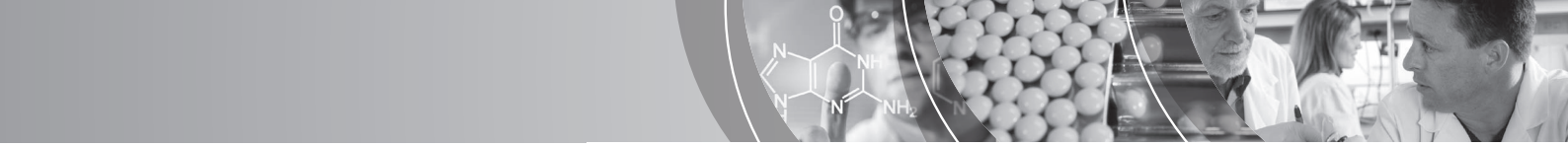
Introduction: The α4β2 nicotinic acetylcholine receptor (nAChR) subtype exists in two stoichiometries, $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$. These receptors have different sensitivities to the natural agonist acetylcholine (ACh) and in the amount of current that pass through each channel. Little is known about the effect of stoichiometry on ligand function, or where on these receptors ligands bind. Recently ACh was found to bind at an additional binding site, located at the α4-α4 interface of the $(\alpha4)_3(\beta2)_2$ subtype [Harpsoe et al, 2011]. This site is relatively unexplored and developing agents that selectively target this site will result in agents that can differentiate between receptor stoichiometries.
Methods: Using the two-electrode voltage clamp technique, we evaluated the antagonists, MLA and a smaller synthetic analog (AE-succinimide) at recombinant rat $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$ nAChRs expressed in the *Xenopus* oocyte system. Using a homology model of the α4-α4 interface, we identified aspartate 204 (D204) located on the complementary side of the α4 subunit that can interact with the succinimide group of MLA and AE-succinimide. To demonstrate that both MLA and AE-succinimide bind to the α4-α4 interface, we attempted to covalently trap the cysteine reactive MLA and AE-maleimide analogs at the two α4β2 receptor stoichiometries containing the α4(D204C) mutation.
Results: We have so far demonstrated that covalent trapping of MLA results in irreversible reduction of ACh-elicited currents in the $(\alpha4)_3(\beta2)_2$ but not the $(\alpha4)_2(\beta2)_3$ stoichiometry [Absalom et al, 2013] indicating that MLA binds to the α4-α4 interface of the $(\alpha4)_3(\beta2)_2$. We are further evaluating whether the smaller AE-succinimide can also bind to the α4-α4 interface.
Conclusion: These studies provide direct evidence of ligand-binding to the α4-α4 interface for a variety of ligands.

Harpsoe et. al., (2011), *J Neurosci*. **31**, pp. 10759-66
Absalom et. al., (2013). *J. Biol. Chem*. DOI:10.1074/jbc.M113.475053



500

Biochemical and functional characterisation of a β2-adrenoceptor multi-protein complex (signalosome)

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Introduction. It was recently discovered that RXFP1, a G-protein-coupled receptor, can be activated with sub-picomolar concentrations of relaxin (Halls et al, 2010). This unusual sensitivity is augmented by the interaction of RXFP1 with several different proteins that together form a constitutively expressed signalosome. These concentrations are within the normal physiological range of relaxin present in the circulation. However, a closely related receptor, RXFP2, does not form a signalosome. Hence, it is important to investigate if this is a common phenomenon among other prototypical GPCRs.
Aims. To investigate if the β2-adrenoceptor can form a signalosome, to identify interacting protein partners, and to pinpoint domains that are important for protein-protein interactions.
Methods. cAMP biosensors transiently expressed in HEK293 cells were used to measure cAMP responses to sub-picomolar concentrations of isoprenaline, mediated by endogenous β2-adrenoceptors. GST immunoprecipitation (IP), co-IP and siRNA knockdown of individual proteins were used to confirm assembly of a signalosome.
Results. β2-adrenoceptor signalosome was activated in HEK293 cells with sub-picomolar concentrations of isoprenaline. Experiments using pharmacological inhibitors showed that cAMP generated by the signalosome is due to $G\alpha_s/G\beta\gamma$ co-activation of adenylyl cyclase, which is dependent on an A-kinase anchoring protein (AKAP). Gαi, PDE4D5 and AKAP79 negatively regulate the signalosome. To confirm the interaction of β2-adrenoceptor with these proteins and identify domains responsible for these interactions, GST IP was performed from HEK293 cell lysates with purified GST-tagged portions of the intracellular loops and the C-terminal tail of the β2-adrenoceptor and analysed by SDS-PAGE and immunoblotting.
Discussion. We show that a β2-adrenoceptor forms a signalosome and can sense sub-picomolar concentrations of ligand. Additional experiments are under way to verify these interactions in model cell lines (HEK293T, CHO-K1) and then in more physiologically relevant system, such as primary cell lines.

Halls ML & Cooper DM (2010) EMBO J 29:2772-87

502

PAR_2-dependent opening of TRPV4 does not depend on PLC activation and Calcium release from stores

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Introduction. We have recently shown that activation of the protease activated GPCR, PAR_2 transactivates the ion channel TRPV4 in HEK293 cells(1). The signalling mechanisms that underlie this transactivation are unknown. The current literature suggests that $G_{\alpha q}$ activation of phospholipase Cβ (PLC) leading to release of calcium from stores is necessary for TRP channel activation and sensitisation.
Aim. To investigate the signalling mechanisms required for PAR_2-dependent activation of TRPV4.
Methods. The rise in intracellular calcium ($[Ca^{2+}]_i$) evoked by PAR_2-actvating peptide (PAR_2-AP) in HEK293 cells was compared in the presence of thapsigargin, $G_{\alpha q}$-blocker (UBO-QIC) and wortmannin, using a FURA-2 calcium influx assay.
Results. PAR_2 activation causes a transient increase $[Ca^{2+}]_i$ in non-transfected HEK293 cells (HEK), however, this activation becomes sustained when TRPV4 is expressed (HEK+TRPV4). This increase in [Ca2+]i is due to influx of Ca^{2+} through TRPV4 (1).The PAR_2-dependent increase in [Ca2+]i from stores can be blocked by Thapsigargin. In HEK+TRPV4 there is still an influx of calcium. This effect is not seen in HEK293 cells that do not express TRPV4. Similarly UBO-QIC dose-dependently blocked the release of calcium from intracellular stores in HEK cells. In TRPV4-HEK expressing cells, the influx of calcium through TRPV4 was not blocked by the UBOQIC. Wortmannin dose-dependently blocked the PAR_2-dependent activation of TRPV4, with an IC_{50} of approximately 1μM.
Discussion. We show that release of calcium from intracellular stores is not necessary for the PAR2-dependent activation of TRPV4. Thus, activation of $G_{\alpha q}$ and PLC is also not crucial for signalling to TRPV4 to open. Wortmannin was used at concentrations at which it is likely to be acting as a non-specific kinase inhibitor, however, its antagonism of the PAR_2 transactivation of TRPV4 shows a link between intracellular kinase signalling as a plausible mechanism for this transactivation.

Poole et al. (2013) JBC 22:288(8):5790-802

504

Identification of receptor-ligand interactions of a novel bitopic ligand at the dopamine D2 receptor

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Introduction: To date, all clinically effective antipsychotics target the dopamine D2 receptor (D2R), competing with the neurotransmitter dopamine for the 'orthosteric' binding site. Unfortunately, this approach is associated with extrapyramidal. Targeting a topographically distinct 'allosteric' site within the receptor may allow for a safe and more selective therapeutic approach. SB269652 was recently identified as the first negative allosteric modulator of D2R. Subsequent ligand fragmentation studies suggested that SB269652 has a 'bitopic' (simultaneous allosteric/orthosteric) mode of interaction with D2R.
Aims: To identify the key receptor-ligand interactions that confer the novel allosteric pharmacology of SB269652 through a combined structure-function and structure-activity approach and to validate a bitopic mode of interaction.
Methods: Homology modelling of the D2R predicted key residues for the binding of SB269652 at both orthosteric and allosteric sites. Residues of interest were mutated to alanine and mutant receptors were stably expressed in FlpIN CHO cells. SB269652 analogues with modifications around three key structural motifs were generated. Radioligand binding and ERK1/2 phosphorylation assays were used to investigate the influence of chemical modifications of the ligand and receptor mutations on ligand affinity and cooperativity.
Results: We identified several residues proposed to sit within a putative allosteric pocket at the extracellular end of transmembrane domains 2 & 7, with SB269652 extending into this region from the orthosteric pocket. Mutation of Glu95 to alanine caused a significant (nine-fold) decrease in affinity and negative cooperativity (five-fold) of SB269652. Homology modelling predicted that Glu95 forms a hydrogen bond with the nitrogen from the indole heterocycle. Methylation of this nitrogen generated an orthosteric antagonist (MIPS1500)(pK_B = 7.28±0.09, Schild slope 0.88±0.12).
Discussion: Structural insights provided by this work provide validation of a bitopic mode of interaction for SB269652. As such they will inform rational drug design, leading to development of improved drugs at this therapeutically important receptor.

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The potential of quinazolines as CYP450 inhibitors and cardiac modulators

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Introduction. Quinazolines are organic compounds that have shown therapeutic potential for a range of targets including DNAPK activity, anti-diabetic effects (Wang & Gao, 2013) and potassium channel modulation (Erb et al, 2000). Hence they may be useful agents for treating cardiac arrhythmia.
Aim. To investigate CYP450 enzyme metabolism inhibition in the presence of the quinazoline analogue LTUJH01 as an initial screening process for drug interactions. Also test the effect of this compound on cardiac contractility.
Method. LTUJH01 is the first of a family of synthesized quinazoline analogues with changes in the benzylamino and pyridine groups on the quinazoline backbone produced in this laboratory. CYP450 enzyme profiling was undertaken using the Baculosome® (Invitrogen) assay for isozymes 3A4, 2C19, 2D6 and 2E1 over a range of LTUJH01 concentrations (10^{-9}Mto 10^{-4}M) with fluorescence measured using a Flexstation 3 (Molecular Devices). Cardiac contractility was studied on isolated whole hearts from snails (*Helix aspersa*) in the presence of LTUJH01, recorded using LabChart® (AD Instruments). Animal experiments were in accordance with ethical approval (AEC06_10BG) La Trobe University. All data were analysed using Graphpad® and Student's unpaired t-test with $\alpha = 0.05$.
Results. LTUJH01 inhibited all CYP450 enzymes tested with average (mean±SEM) $\log_{10}IC_{50}$: 5.3±0.2(3A4, n=14), 5.1 ±0.1(2C19, n=12), 5.5±0.8(2D6, n=10) and 6.9±0.1(2E1, n=17), respectively. For CYP2E1 the compound LTUJH01 was more potent than the reference inhibitor (cupral, $\log_{10}IC_{50}$ 4.3±0.1, n=6, $P=2x10^{-22}$). Both force ($\log_{10}IC_{50}$=7.9±0.5, n=6) and heart rate ($\log_{10}IC_{50}$=6.9±0.5, n=6) were decreased in the presence of LTUJH01.
Discussion. LTUJH01 decreases cardiac contractility (heart rate and force) in a dose-dependent manner. It is a potent inhibitor of CYP450 enzymes commonly involved in metabolizing cardiac medicines and may potentially cause drug interactions in polypharmacy.

Erb B et al (2000) J Het Chem, 37**:** 253-260
Wang D & Gao F (2013) Chem Cen J 7**:** 95-99

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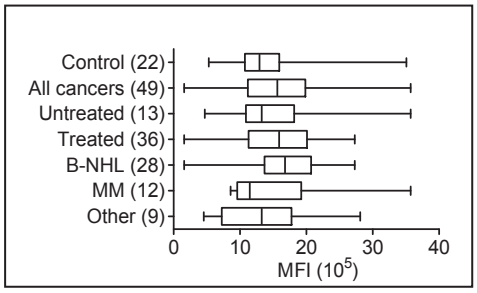
TRPV1 expression in haematological malignancies

Sofia Omari, Murray J Adams, Alhossain A Khalafallah, Muhajir Mohamed, Dominic P Geraghty. School of Human Life Sciences, Univ of Tasmania, Launceston, TAS.

Aim. Transient receptor potential vanilloid-1 (TRPV1) is a non-selective cation channel activated by a variety of endogenous and exogenous stimuli. Overexpression of TRPV1 is associated with several cancers. The aim of this study was to investigate TRPV1 expression in haematological malignancies.



Methods. TRPV1 expression was measured using flow cytometry (Attune® Acoustic Focusing Cytometer (Life Technologies)) and Western blotting in 49 patients (Ethics Approval H11050). The patient population (age 31-85 yrs; M:F, 28:21) was divided into three groups: B-cell Non-Hodgkin's lymphoma (B-NHL, 28), multiple myeloma (MM, 12) and others (9, comprising myeloproliferative disorder (MPD, 2), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS, 1), and other leukaemias (6)). Blood and protein samples from gender-matched healthy controls (n=21) were analysed in parallel with patient samples.

Results. TRPV1 was detected in all patients and controls using flow cytometry. TRPV1 expression in patients was similar to healthy controls (shown as the median of mean fluorescence intensity, MFI, with bars representing range). There were no significant differences in TRPV1 expression between MM and B-NHL patients, or between newly-diagnosed (untreated) patients and those undergoing treatment. Using Western blotting, TRPV1 (~97kDa band) was only detected in 1 MM and 4 B-NHL patients. A 240kDa band was also detected in 1 B-NHL and 1 MM patient.

Discussion. To our knowledge, this is the first study to report TRPV1 expression in blood cells from haematological malignancies. In contrast to other cancers, TRPV1 is not overexpressed in malignant blood cells, and is expressed to a similar extent in B-NHL and MM patients.

Supported by the Clifford Craig Medical Research Trust

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From structure to function: understanding the role of a highly conserved intramembranous binding pocket at GPCRs.

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Introduction. G protein–coupled receptors (GPCRs) are the major mediators between extracellular stimuli and intracellular outcomes, in living organisms. Unfortunately, these receptors often display high sequence homology within subtypes, limiting drug selectivity. To overcome this major drawback, a greater understanding of the structural basis of these receptors is required. Recent advances in several technological developments have led to the successful crystallization of Family A GPCRs, especially in the muscarinic acetylcholine receptor (mAChR) family. Interestingly, a number of residues, creating an intramembranous binding pocket located immediately below the orthosteric binding site of the M_2 mAChR, have been identified. This pocket, made of highly conserved residues across family A receptors, may be general to most inactive GPCR structures.

Aims. This intramembranous pocket may play an important structural and functional role of GPCRS, and may represent potential unappreciated novel drug-binding sites. We decided to investigate the role(s) of this highly conserved intramembranous pocket at GPCRs, using the M_2 mAChR as a template.

Methods. We generated alanine mutations of 6 specific residues: $L65^{2.46}$, $S107^{3.36}$, $L114^{3.43}$, $I392^{6.40}$, $F396^{6.44}$, and $N432^{7.45}$, and investigated the role of each individual residues at both the level of binding and the level of function.

Results. Performing [^{3}H]NMS radioligand binding experiments, we estimated the affinity constant (K_D) of ten structurally distinct muscarinic ligands: agonist, antagonist, allosteric and bitopic ligands. Secondly, we determined the ability of these ten ligands to signal through the different receptor mutants, performing ligand-mediated extracellular signal-regulated kinases (ERK) 1/2 assays.

Discussion. The outcome of this study is that specific residues located in this intramembranous pocket are important for the structure and function of GPCRs. In particular, we demonstrated that $L114^{3.43}$, $I392^{6.40}$ and $F396^{6.44}$ are involved in the stabilisation of an inactive conformation of the M_2 mAChR.

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Expression of known and novel androgen receptor splice variants in human tissues and breast cancer cells

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Introduction. Androgen receptor splice variants (ARVs) that lack the carboxyl-terminal ligand-binding domain play a role in castration-resistant prostate cancer development and are regarded as novel factors that mediate resistance to androgen depletion therapy. The androgen receptor contains 8 canonic exons and 5 cryptic exons. The splicing of a cryptic exon to an upstream canonic exon generates at least 14 ARVs (V1 to V14) in normal and cancerous prostate tissues, six of which (V1, V3, V4, V7, V9, and V12) are shown to be active transcription factors. Whether these active ARVs are expressed in human tissues other than the prostate and their potential role in other steroid sensitive cancers (e.g., breast cancer) remain unexplored.
Aims. To define the expression profiles of active ARVs in human tissues and breast cancer cell lines.
Methods. A panel of 21 human tissues and a panel of 10 human cell lines including 6 breast cancer cell lines were screened by ARV-specific quantitative real-time reverse transcriptase (RT) PCR. RT-PCR products were cloned and sequenced. The function of AR-V7 in breast cancer cells was assessed by luciferase AR reporter assays.
Results. Transcripts of four active ARVs (V1, V3, V7, and V9) were detected in almost all of the screened human tissues and cell lines. Overexpression of V7 in breast cancer cells stimulated AR-responsive reporter activities. We discovered four novel ARVs in breast and prostate cancer cell lines and one novel single nucleotide polymorphism encoding a variant for AR-V7, an important ARV in prostate cancer.
Discussion. We provide evidence for the first time that ARVs are widely expressed in human tissues and breast cancer cells. As the AR is widely expressed in human tissues and has biological function in many human organs, our observations highlight the potential pathological implications of ARVs underlying human diseases other than prostate cancer.

Dehm SM et al (2011) Endocr Relat Cancer 18:R183-196
Matsumoto TM et al (2012) 75:201-224

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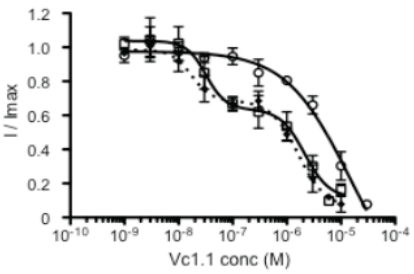
A novel, high affinity binding site in α9α10 nicotinic acetylcholine receptor

Dinesh C. Indurthi[1], Nathan L. Absalom[1] and Mary Chebib[1]. Faculty of Pharmacy[1], University of Sydney, Sydney, NSW.



Introduction: Nicotinic acetylcholine receptors (nAChR) are members of Cys-loop family of ion channels and are implicated in several nervous system disorders [Gotti et. al., 2006]. The α9α10 nAChR subtype is recently known to be involved in immune-modulation, along with its well-known role in the auditory system. Antagonists of α9α10 nAChR like Vc1.1, an α-conotoxin isolated from the marine snail, Conus *victoria*, are thought to mediate their analgesic effect partly via this receptor [Satkunanathan et al., 2005].
Methodology: We investigated the involvement of two different stoichiometries of α9α10 receptors, in heterologously expressed in *Xenopus* oocytes, on ACh activation and Vc1.1 inhibition of ACh-evoked currents. We altered the injection ratio of α9 and α10 subunit mRNA to alter receptor stoichiometry. A Student's t-test was performed to evaluate differences in IC_{50} of Vc1.1 obtained from the two injection ratios.
Results: A bi-phasic ACh induced excitation curve was observed when oocytes were injected with excess α9 compared to α10 mRNA, in contrast to the monophasic curve observed when excess α10 mRNA was injected. We observed a similar trend in the inhibitory curve of Vc1.1 as shown in the figure above. The IC_{50} values were 32nM and 1.6μM for α9:α10 injection ratio of 10:1, compared to IC_{50} of 4.67 μM in 1:3 injection ratio.
Conclusion: These results highlight differences in the pharmacological profile of α9α10 nAChR stoichiometries, hypothesized to be $(\alpha9)_3(\alpha10)_2$ and $(\alpha9)_2(\alpha10)_3$, that are controlled by the injection ratios. We infer that there is a high affinity Vc1.1 binding site on $(\alpha9)_3(\alpha10)_2$ nAChR receptor, and suggest this to be at the α9-α9 interface, in addition, there is a low sensitive binding site at the α10-α9 interface that is common to both stoichiometries. This is the first study to highlight the presence of two different binding sites for Vc1.1 on α9α10 nAChR.

Gotti et.al., (2006) TiNS,. **27**(9): 482-491.
Satkunanathan et al., (2005) Brain Research,. **1059**(2): 149-158

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In vitro screening of novel P2X7 receptor antagonists with potential antidepressant activity

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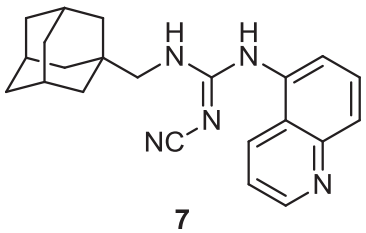
Introduction. Neuroinflammation may be part of the pathophysiology of depression. Since activation of the P2X7 receptor leads to release of inflammatory mediators from microglia, antagonists of the P2X7 receptor may be effective anti-neuroinflammatory, and hence antidepressant, drugs.

Aims. To characterise the functional activity and cytotoxicity of a series of cyanoguanidine-derived compounds based on the structure of a known P2X7 receptor antagonist, 2-cyano-1-[(1S)-1-phenylethyl]-3-quinolin-5-ylguanidine (A804598).



7

hP2X7 pIC50 = 8.55

Methods. The cyanoguanidine-derived lead compound A804598 and six structurally similar compounds were assayed for functional activity at the P2X7 receptor. The ability of the compounds to inhibit agonist-induced, cytolytic-pore formation in THP-1 cells was measured using a dye uptake assay. The compounds were further characterised by their ability to inhibit agonist induced interleukin-1ß (IL-1ß) release. The cytotoxicity of the seven compounds was also evaluated using a viability assay reliant on cellular metabolism in which THP-1 cells were exposed to 10 μM of test compound for 24 h.

Results. All seven compounds behaved as antagonists at the P2X7 receptor in the dye uptake assay. The pIC50 values ranged from 5.68 to 8.55. The compounds displayed the same rank order of potency in the inhibition of agonist induced IL-1ß release. None of the compounds displayed cytotoxicity.

Discussion. Substitution of the 1-phenylethyl group (A804598) for an adamantan-1-ylmethyl group (7) lead to greater than a 10 fold increase in potency, representing the most useful finding from this series of compounds. The absence of toxicity exhibited by these compounds was also encouraging, but more complex cellular models of toxicity need to be employed to confirm this property. Empirical Log P determination of A804598 and 7 should follow to allow calculation of lipophilic efficiency. Through optimisation of potency and predicted CNS penetrance, suitable candidates for *in vivo* testing may be selected.

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Altered trafficking profiles of angiotensin II receptor heteromers

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Introduction. The vasoconstrictive hormone angiotensin II acts upon two different G protein coupled receptors (GPCRs), the angiotensin II type 1 receptor (AT_1R) and the angiotensin II type 2 receptor (AT_2R). Almost all of the characteristic biological actions of angiotensin II, are mediated by the AT_1R. In contrast, the molecular and physiological functions of the AT_2R remain poorly understood, though it is often believed to counteract many AT_1R-mediated effects. Like many GPCRs, the concept of receptor heteromerisation has revealed new complexity in angiotensin II receptor signalling systems through the formation of receptor complexes with unique pharmacological profiles.

Aims. The aim of this study was to compare the internalisation and trafficking profiles of various angiotensin II receptor complexes with that of the receptor monomer/homomers.

Methods. Receptor internalisation and trafficking were investigated using an unconventional approach employing bioluminescence resonance energy transfer (BRET). In this study, receptor proteins were tagged with the Rluc8 luciferase donor enzyme, while plasma membrane and endocytotic protein markers were tagged with the Venus fluorophore. Changes in the BRET signal upon ligand treatment enabled monitoring of cellular localization of the receptors. Addition of a second, untagged receptor into the system allowed comparisons between monomer/homomer and heteromer trafficking profiles.

Results. The results of this study have revealed that angiotensin II receptor heteromers have different internalisation and trafficking profiles from that of the monomeric/homomeric receptors.

Discussion. This study has demonstrated the use of a novel approach for investigation of receptor internalisation and trafficking. Additionally it has enabled characterisation of the cellular trafficking profiles of various angiotensin II receptor heteromers.

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A structure-activity analysis of biased agonism at the dopamine D_2 receptor

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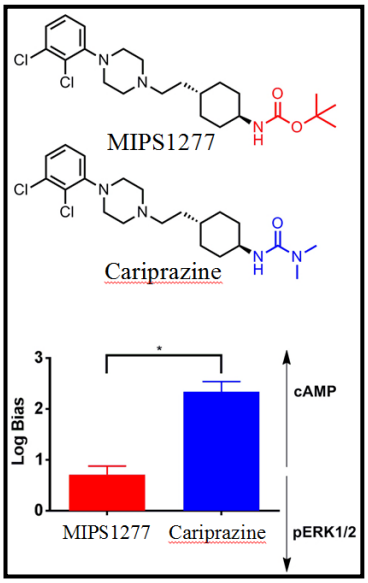
Introduction. Biased agonism is the ability of ligands acting at the same GPCR to stabilize distinct receptor conformations that, in turn, are linked to different functional outcomes. A number of studies have suggested that biased agonism at the dopamine D_2 receptor (D_2R) may be advantageous for the treatment of neuropsychiatric disorders, including schizophrenia. As such, it is of great importance to gain insight into the structure-activity relationship (SAR) of biased agonism at this receptor.

Aims. The aim of our study was to generate SAR around a novel D_2R partial agonist, *tert*-butyl (*trans*-4-(2-(3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)carbamate. This ligand shares structural similarity to cariprazine, a drug awaiting FDA approval for the treatment of schizophrenia, yet displays a distinct bias towards two different signaling endpoints.

Methods. We synthesized derivatives focused on three main portions of the lead compound: the tertiary amine-containing "head group"; the cyclohexylene "spacer" group, and the *tert*-butyl carbamate "tail group". We combined this approach with novel analytical pharmacology methods that allow us to quantify biased agonism and gain novel insight around SAR for the fine control of ligand efficacy and biased signaling at the D_2R.

Discussion. We have demonstrated that efficacy and biased agonism can be finely tuned by minor structural modifications to (1) the head group containing the tertiary amine, (2) a tail group that extends away from this moiety and (3) the orientation and length of a spacer region between these two moieties.



514

Probing the binding of allosteric modulators at the human calcium sensing receptor (CaSR)

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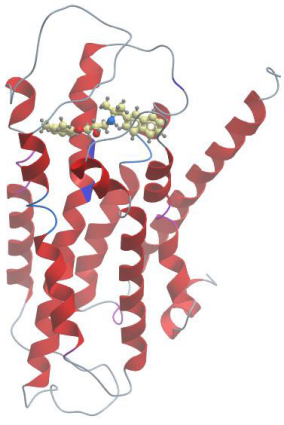
Introduction. More than 200 naturally occurring mutations have been identified in the human CaSR, which cause disorders of calcium homeostasis such as autosomal dominant hypocalcaemia, Bartter's syndrome type V, familial hypocalcuiric hypercalcalcaemia and neonatal severe hyperparathyroidism. Many of these mutations are located in receptor regions that are predicted to form the binding site for small molecule drugs such as cinacalcet, which has been used clinically to correct calcium disregulation in patients carrying "loss-of-function" mutations. It is thus imperative that we understand where cinacalcet and other small molecule drugs bind in the CaSR, so that we can predict how patients harbouring mutations will respond to small molecule drug therapy that can correct mutation-induced impairments in receptor function.

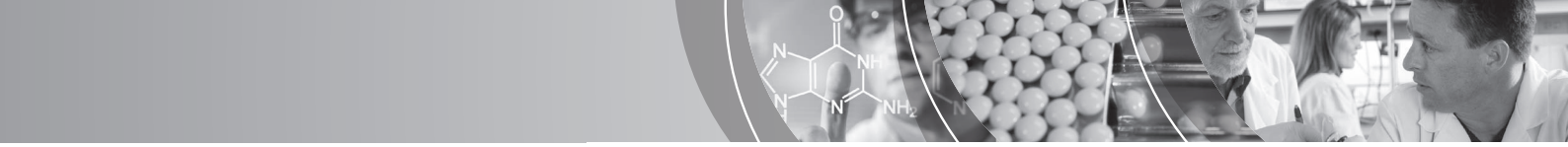


Aims. To probe the binding site for cinacalcet in the human CaSR.

Methods. Amino acid substitutions were introduced into the CaSR's transmembrane domains (TM). Substitutions were chosen based on previously predictions from molecular modelling studies of amino acid residues that contribute to the binding pocket of small molecule drugs. In addition the effects of naturally occurring mutations were examined. The effect of mutations on the binding affinity of cinacalcet was determined using functional interaction experiments.

Results. Mutations at the majority of the residues predicted to contribute to the cinacalcet binding pocket located throughout the TM domains of the CaSR had no effect on the binding affinity of cinacalcet. Similarly, many naturally occurring mutations in the TM regions did not alter cinacalcet binding. However, a cluster of residues at the top of TM2 reduced the binding of cinacalcet.

Discussion. Previous predictions of the CaSR structure and cinacalcet binding site require refinement. The majority of patients with naturally occurring mutations in the CaSR's TM domain would benefit from treatment with small molecule drugs because most mutations are unlikely to disrupt the binding of these drugs.



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The Synthesis and Biological Study of Curcumin Analogues as Anticancer Agents

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Introduction. Curcumin, the major bioactive constituent of turmeric holds great therapeutic potential for the treatment of cancer as it displays activity against various types of cancers, both *in vitro* and *in vivo*. More importantly, curcumin has no known toxicities and to date no maximum tolerated dose has been determined.
The anticancer properties of curcumin have been attributed to its ability to interfere with a diverse range of molecular targets in multiple pathways involved in cancer, and it could therefore be more efficacious in comparison to current mono-targeted anticancer therapies (Anand et al, 2008). Despite its promising anticancer properties, the clinical application of curcumin is limited due to its poor stability, bioavailability and metabolic profile (Pan et al, 1999).
Aims. To improve the physicochemical, pharmacokinetic profiles and anticancer activity of curcumin by synthesising analogues of curcumin.
Methods. Curcumin analogues were synthesised *via* aldol condensation reaction of diketones with substituted benzaldehydes. The analogues were screened for anticancer activity using MTT assay and cell cycle analysis were performed on selected analogues.
Results. Fifty curcumin analogues were screened in PC3 prostate cancer cell line using the MTT assay and six analogues showed promising anticancer activities. Cell cycle analysis was conducted and the six analogues caused G_2/M cell cycle arrest in PC3 cell line, without induction of apoptosis.
Discussion.The anticancer property of curcumin analogues have better anticancer properties compared to curcumin in this study. A structure-activity relationship model will need to be constructed to assist with designing of new analogues in the future.

Anand P et al (2008) Cancer Lett, 267:133-164.
Pan MH, Huang TM, Lin JK. (1999) Drug Metab Dispos. 27:486-94.

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Design and testing of novel anti-cancer agents targeting secretory pathway calcium ATPase

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Introduction. Basal-like breast cancer has the poorest prognosis of all known breast cancer subtypes and there are no effective targeted treatment agents currently available. Secretory pathway calcium ATPase (SPCA) has been found to relate to tumour growth in basal-like breast cancer and may be a potential drug target.
Aims. To design and test novel chemical compounds that will inhibit SPCA.
Methods. *In silico* molecular modelling was used to generate an SPCA model based on its 1d isoform, and four potential drug binding sites were found. This was followed by a rational drug design process and compounds selected on predicted binding affinity were tested *via* fluorometric imaging plate reader (FLIPR) to measure intracellular calcium level in the MDA-MB-231 cell line. A similar process was used to design a compound inhibiting sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA).
Results. One of the compounds tested in the basal-like breast cancer cells might be a weak SPCA inhibitor. The compound designed to target SPCA1d demonstrated modest effects on intracellular calcium compared to the control compound cyclopiazonic acid (CPA). A second compound designed to target SERCA also showed minimal effects when compared to CPA.
Discussion. Two novel compounds were designed and tested in the breast cancer cell line. The results obtained from FLIPR indicated that both compounds were likely weak inhibitors of both SPCA1d and SERCA. To improve the activity of SPCA1 inhibitors, it may be necessary to use similar core structural motifs present in CPA.

Badve S et al (2011) Mod pathol 24(2):157-67
Bayraktar S et al (2012) Breast cancer res treat 135(2):355-66

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A switched on receptor - constitutive activity of the adenosine A_{2B} receptor

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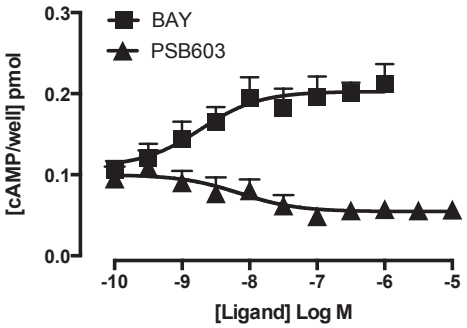
Introduction. Constitutive activity is defined as the spontaneous isomerization of a receptor to an active confirmation that results in a cellular response. High basal cAMP concentrations within a heterologous expression system suggest the adenosine A_{2B} receptor (A_{2B}AR) may be highly constitutively active.

Aims. To profile A_{2B}AR constitutive activity across multiple signal transduction pathways in both a heterologous expression system and in prostate cancer cell lines that endogenously express A_{2B}AR.

Methods. The influence of prototypical A_{2B}AR agonists, adenosine, NECA and BAY60-6583 and classical antagonists ZM24138, PSB603 and DPCPX was profiled across different signal transduction pathways including cAMP accumulation, pERK 1/2 and IP-One accumulation in FlpINCHO cells stably expressing the human A_{2B}AR. Subsequent studies investigated the same ligands in the endogenous expression system, the 22RV1 cells in the cAMP-signalling assay.



Results. In the absence of endogenous adenosine, the A_{2B} antagonists, ZM24138, PSB603 and DPCPX all mediated a concentration dependent decrease in baseline basal cAMP levels. In contrast, despite the presence of A_{2B}AR agonism, this decrease below baseline was not observed in either pERK1/2 or the IP-One assay. A similar concentration dependent decrease in cAMP was observed in the 22RV1 cells in the presence of inverse agonists

Discussion. Constitutive activity of the A_{2B}AR was clearly observed down the G_s-coupled cAMP pathway in both the A_{2B} FlpINCHO cell line and the prostate cancer cell line, 22RV1. Given the A_{2B}AR has been implicated in mediating cell proliferation of many solid tumours, decreasing constitutive activity with inverse agonists may be useful in reducing tumour cell growth.

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Evaluation of the biological activity of novel trishomocubanes compounds at sigma receptor binding sites

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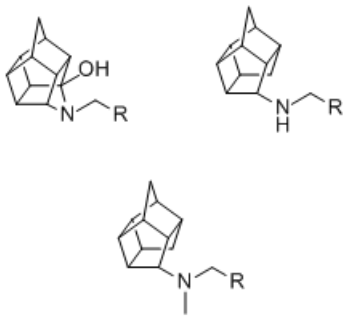
Introduction. Sigma receptors are unique mammalian proteins, widely distributed in the CNS and periphery, with two defined subtypes (σ-1 and σ-2). We have developed a library of trishomocubanes with σ receptor affinity, and identified structural motifs that confer subtype selectivity.

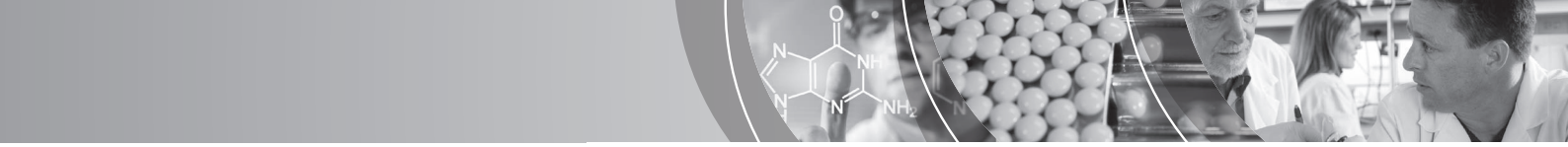


Aim. To further explore the pharmacological profile of trishomocubane σ receptor ligands by investigating their potential neuroprotective activity and cytotoxicity.

Method. The ability of 18 trishomocubanes to inhibit nitric oxide (NO) production in lipopolysaccharide stimulated RAW264.7 cells was evaluated using a Griess assay. The test compounds were also screened in a resazurin-based cytotoxicity assay. The selective σ-1 ligand 4-phenyl-1-(4-phenylbutyl) piperidine (PPBP) was screened as a reference.

Results. Nine trishomocubanes showed significant dose-dependent inhibition of NO production equal to, or greater than, PPBP. At the highest concentrations evaluated, some trishomocubanes displayed cytotoxicity.

Discussion: The selective σ-1 agonist PPBP suppresses the production of the neuroinflammatory mediator NO and demonstrates marked neuroprotective activity in animal models of stroke.. Our results show, for the first time, that trishomocubane σ receptor ligands, including those with preferential binding to σ-2 receptors, are able to significantly inhibit NO release.



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Structure-function analysis of allosteric ligand binding at the adenosine A_1 receptor.

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Introduction. The adenosine A_1 receptor (A_1AR) represents a therapeutic target for a variety of disorders. Allosteric modulators of the A_1AR interact with a topographically distinct binding site from that of the endogenous ligand. Allosteric enhancers of the A_1AR offer a number of potential advantages including increased subtype selectivity and preservation of the spatial and temporal pattern of endogenous agonist signalling. The development of A_1AR therapeutics would benefit immensely from the rational design of more selective and efficacious A_1AR allosteric ligands, however this approach requires greater structural knowledge of the A_1AR allosteric binding site.
Aim. To probe the key residues involved in conferring A_1AR allosteric ligand affinity, efficacy and cooperativity.
Methods. Homology modelling predicted key residues involved in allosteric ligand binding at the A_1AR. Mutant A_1ARs containing a single alanine substitution were stably expressed in FlpINCHO cells. Interaction studies between the orthosteric ligand, NECA, and the allosteric ligands, VCP171 and PD81723, were used to quantify the influence of receptor mutations on the allosteric ligand affinity, efficacy and cooperativity in radioligand binding and cAMP accumulation assays.
Results. Three transmembrane mutations, V87A, Q92A, and N184A, significantly decreased, when compared to the wild-type A_1AR, the positive binding cooperativity observed between NECA and PD81723 ($n=4$; $p<0.05$). Similarly, the mutations, V87A and Q92A, significantly reduced the functional cooperativity between NECA and PD81723, whereas the mutations, Q92A and N184A, significantly reduced the functional cooperativity between NECA and VCP171 ($n=4$; $p<0.05$).
Discussion. At the A_1AR, V87, Q92 and N184 play an important role in conferring conformational rearrangements upon receptor activation. This may explain the influence of these residues, when mutated to alanine, on the transmission of binding and functional cooperativity between the orthosteric and allosteric sites.

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Calcium transporters and modulator profiling in trastuzumab-resistant SKBR3R breast cancer cells

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Introduction. HER2-positive breast cancers are approximately 20% of all breast cancers and are characterised by an overexpression of the growth factor receptor HER2. Trastuzumab, a monoclonal antibody, is a molecularly targeted therapeutic used in the treatment of this subtype of breast cancer. However, 30% of eligible patients have intrinsic resistance to trastuzumab while most patients who initially response to the therapeutic develop resistance within one year. Calcium transporters and modulators are known to be involved in breast cancer and in chemoresistance. However, their role has not been evaluated in HER2-positive trastuzumab-resistant breast cancer cells.
Aims. To establish trastuzumab-resistant HER2-positive breast cancer cell lines and compare the mRNA levels of calcium transporters and modulators in trastuzumab resistant and sensitive SKBR3 cell lines.
Methods. MTS assays were used to assess sensitivity to trastuzumab and identify resistant cell lines. Real-time RT-PCR was used to evaluate mRNA levels.
Results. Parental HER2-positive SKBR3 cells were continuously cultured for seven months in the presence or absence of trastuzumab (10 μg/mL) to establish trastuzumab-resistant and aged match control cell lines. At the end of the seven months resistance protocol two trastuzumab-treated colonies showed resistance to trastuzumab and two colonies from the aged match control group showed the acquisition of de-novo resistance. The mRNA expression of 43 targets including Ca^{2+} pumps, channels and channel modulators and the growth factor receptors HER2 and EGFR, were evaluated in trastuzumab-resistant and sensitive cell lines. While all cell lines maintained HER2 receptor overexpression some changes were observed in the expression of specific calcium transporting proteins.
Discussion. The results of these studies suggest that the acquisition of trastuzumab resistance may be associated with an alteration in the expression of specific channels and pumps.

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Activation of over-expressed calcium channels as a potential therapeutic strategy for the treatment for breast cancer

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Introduction. Elevated expression of calcium channels is a feature of some cancers including those of the breast. Numerous studies show that inhibition of some overexpressed calcium channels in cancer cells can inhibit proliferation and/or metastasis. Activation of over-expressed calcium channels is also suggested as an alternative strategy for the treatment of cancer, as increases in cytosolic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) are associated with the induction of cell death, however, activation could potentially promote proliferation. A breast cancer cell line with inducible TRPV1 overexpression was used as a model to assess the potential for ion channel activators as therapies for breast cancer. TRPV1 is a calcium permeable ion channel that is activated by capsaicin, the hot component of chilli peppers.
Aims. To determine the relationship between TRPV1 expression and activation on the proliferation and viability of MCF7 breast cancer cells
Methods. Ca^{2+} assays using a fluorescent imaging plate reader (FLIPR) were used to assess Ca^{2+} responses to capsaicin in MCF7 cells with inducible TRPV1 expression ($MCF7^{TRPV1}$). MTS assays were used to assess the effect of TRPV1 overexpression and capsaicin on proliferation and viability of $MCF7^{TRPV1}$ cells. Cell death was assessed using propidium iodide and YO-PRO-1 staining. Real-time RT-PCR was used to evaluate mRNA levels.
Results. Capsaicin did not induce proliferation of $MCF7^{TRPV1}$ cells at any level of TRPV1 expression. Capsaicin reduced the viability of $MCF7^{TRPV1}$ cells at high TRPV1 expression levels in a concentration-dependent manner. Both over-expression and activation of TRPV1 were required to induce cell death. Capsaicin induced expression of the early response gene, c-fos, when TRPV1 expression was induced.
Discussion: These findings suggest that breast tumours that overexpress TRP channels may be therapeutically targeted using specific pharmacological activators.

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A flavonoid rich extract from *Carpobrotus rossii* protects against glucose intolerance

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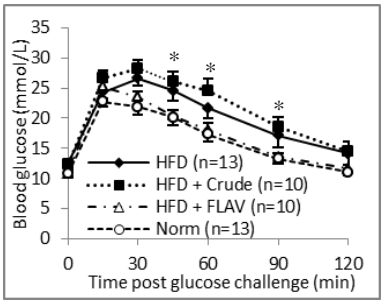
Introduction. Glucose intolerance and insulin resistance are dominant features in the development of type II diabetes and/or metabolic syndrome (Cersosimo and DeFronzo, 2006). A crude extract from the native plant *Carpobrotus rossii* (CR) has been shown to have lipid lowering actions (Geraghty et al., 2010).
Aims. To determine whether consumption of CR extracts improves glucose tolerance in insulin resistant mice.
Methods. Male C57/BL6 mice (6 wks) were fed either a normal (Norm, 9% fat w/w) or high fat (HFD, 22% fat w/w) diet to induce insulin resistance. Two of the three groups of HFD mice were supplemented with either crude CR extract (HFD + Crude) or a refined, CR flavonoid-rich extract (HFD + FLAV) in their drinking water. HPLC analysis ensured that both CR groups received an equivalent mg/kg flavonoid dose. After 28 days, mice were fasted for 6 h and injected with 2 g/kg glucose i.p. Their blood glucose levels were monitored every 15-30 min for 2 h.
Results. HFD + FLAV supplemented mice had significantly lower blood glucose levels at 45, 60, and 90 min following the glucose challenge (all $P<0.05$), compared with HFD controls. The CR + FLAV group had similar glucose clearance to the Norm group. The HFD + Crude group's glucose clearance was no different from the HFD group.
Discussion. Earlier work in healthy rats found that crude CR extract has lipid-lowering activity. CR flavonoids, but not the crude extract, were responsible for improved glucose tolerance. Investigation to determine the effects of the CR flavonoids on other aspects of metabolic syndrome (hyperlipidaemia, hypertension) are recommended.



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Geraghty DP et al, (2010), Proceedings of ASCEPT, p.36.

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Elucidating the pharmacological signalling pathways underlying beta-adrenoceptor regulation of breast cancer metastasis

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Introduction. Chronic stress has been demonstrated to increase metastasis to distant tissues from primary mammary tumours and these effects can be completely reversed in the presence of a beta-adrenoceptor (beta-AR) antagonist, propanolol. Pharmacologic activation of beta-AR signalling with a beta-AR agonist, isoprenaline, was able to mimic the effects of chronic stress. This highlights the importance of beta-AR signalling in regulating metastasis and suggests that beta-blockers may have potential in adjuvant therapy to slow breast cancer progression (Sloan et al, 2010).
Aims. To identify the signalling pathways that mediate beta-AR regulation of metastasis.
Methods. MDA-MB-231 human breast cancer cells were treated with a range of beta-AR agonists and antagonists. Population based signalling assays were performed to measure a variety of intracellular signalling mediators including pERK1/2, cAMP, mTOR, pSTAT and p38 levels.
Results. Beta-AR agonist isoprenaline and beta2-AR specific agonists salbutamol and formoterol inhibited phosphorylation of ERK1/2 in a time-dependent and dose-dependent manner. After 15 min of treatment, isoprenaline, salbutamol and formoterol inhibited ERK1/2 phosphorylation with pIC50s of 6.95+/-0.16 (100 nM), 9.72+/-0.07 (0.1 nM) and 10.93+/-0.06 (0.01 nM), respectively. In contrast, beta1-AR agonists xameterol and RO363 did not modulate ERK1/2 phosphorylation. ICI 118551, a beta2-AR specific antagonist reversed isoprenaline, salbutamol and formoterol inhibition of ERK1/2 phosphorylation to a greater extent than propanolol, a beta-AR general antagonist and CGP 20712A, a beta1-AR specific antagonist. However, the antagonists did not modulate ERK1/2 phosphorylation in the absence of agonist.
Discussion. This study has found that pharmacologic activation of beta2-AR signalling and not beta1-AR in human breast cancer cells is inhibitory to ERK1/2 phosphorylation. Given the central role of ERK phosphorylation in MAPK signalling, our results suggest that this pathway may have a major role in metastasis and cancer progression.
Sloan E et al (2010) Cancer Res 70: 7042-7052.

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Serum-Induced SIRT1 Expression and Longevity in Older Men

Shajjia Razi[1,2,3], Vicky L. Benson[2], Victoria Cogger[1,2,3], Vasi Naganathan[1,2], Aisling McMahon[1,2], David Le Couteur[1,2,3], Rafa de Cabo[5], David G. Hadelsman[1,2]. Anzac Research Institute[1], Concord, NSW; The University of Sydney[2], Sydney, NSW; Centre for Education and Research on Ageing[3]; Concord Repatriation and General Hospital[4], Concord, NSW, Australia; National Institute of Aging[5], Baltimore, United States of America.

Introduction. Caloric restriction influence circulating factor that have an effect on SIRT1 expression.
Aims. To determine the association between frailty and such circulating factors, we measured serum-induced SIRT1 expression from nested cohort of frail (n=77) and robust (n=82) participants from The CHAMP study.
Methods. Concord Health Ageing in Men Project is a population based longitudinal study of community-dwelling men older than 70 years. Serum-Induced SIRT1 expression is measured using a modified read out indirect ELISA bioassay.
Results. Serum-Induced SIRT1 expression was not different between frail and robust men (103.1±17.0 versus 100.4±19.3 ug/L). However subsequent analysis showed that men with the lowest value of serum-induced SIRT1 expression were less likely to be frail (p=0.04).
Discussion. We further measured serum-induced SIRT1 expression from CHAMP cohort of frail and pre-frail (n=573) and robust (n=545). Serum-induced SIRT1 expression was not different between frail and robust men (113±30 versus 109±30 ug/L) with a p value of 0.06. Nonetheless following analysis exhibited that serum-induced expression at baseline predicts mortality as men with highest SIRT1 expression have shortest survival. The serum factors that mediate sirtuin expression are still unknown.

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Investigating recruitment of regulatory proteins to the GLP-1R and their role in downstream signaling

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Introduction. The glucagon-like peptide-1 receptor (GLP-1R) a class B G protein-coupled receptor (GPCR) responds to multiple endogenous ligands including four variants of GLP-1. This receptor is also activated by the exogenous peptide exendin-4 and allosteric ligands. The GLP-1R is essential in nutrient regulated insulin release, therefore a therapeutic target for treatment of type II diabetes mellitus and obesity.
Insulin secretion downstream of GLP-1R activation is critically dependent on cAMP formation, but recent evidence suggests an essential role of regulatory proteins such as β-arrestins (β-Arr1) and G protein-coupled receptor kinases (GRKs). The canonical role of these regulatory proteins is to terminate GPCR signaling and promote receptor internalization. However, more recently, roles as scaffolding proteins that can regulate G protein-independent signaling have emerged (Gurevich et al., 2012)
Aim. This work investigates the role of regulatory proteins in GLP-1R function.
Method. We have applied bioluminescence energy transfer to measure agonist-induced recruitment of β-Arr1, β-Arr2, GRK2, -3, -5 and -6 to the GLP-1R in a ChoFlpIn cell background.
Results. This established recruitment profiles of these regulatory proteins for multiple peptide and non-peptide ligands. The ability of the allosteric ligands, Compound 2 and BETP, to modulate orthosteric ligand-mediated profiles was also assessed. This revealed βArr1, βArr2, GRK2 and -3 (but not GRK5 or -6) recruitment by GLP-1(7-36)NH2, exendin-4 and oxyntomodulin could be positively modulated by both classes of compounds (albeit weakly by BETP), but the degree of modulation varied depending on the orthosteric ligand present. Additionally, to assess the physiological role of each of these regulatory proteins we have used shRNA to specifically knock down their expression individually in a insulinoma cell line and measured the effects on GLP-1R mediated signaling pathways after treatment by different ligands.
Discussion. These data provide further insight into the cellular mechanisms of GLP-1R action.

Gurevich E.V. et al., (2012) Pharmacol Ther. 133(1):40-69

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Evaluation of the potential of *Syzygium australe* and *Syzygium leuhmannii* fruit extracts as antibacterial agents

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Introduction. Many species of Syzygium are known to have antiseptic activity. Several Australian Syzygium species had roles as traditional bush medicines for Australian Aborigines although their antiseptic potential has not been rigorously studied.
Methods. The antimicrobial activity of solvent extracts of *Syzygium australe* and *Syzygium leuhmannii* fruits were investigated by disc diffusion assay against a panel of bacteria and fungi and their MIC values were determined. Toxicity was determined using the *Artemia franciscana* nauplii bioassay.
Results. The methanolic extracts of the fruit of both Syzygium species displayed the greater antibacterial activity of the extracts tested. *S. australe* generally had greater efficacy than the *S. leuhmannii* extracts. *S. australe* and *S. leuhmannii* fruit methanolic extracts inhibited the growth of 13 (93 %) and 12 (86 %) of the 14 bacteria tested respectively. Gram-positive and Gram-negative bacteria were both susceptible, although a slightly greater susceptibility of Gram-positive bacteria was noted. Nine (90 %) and 8 (80 %) of the 10 Gram-negative bacteria had their growth inhibited by *S. australe* and *S. leuhmannii* fruit methanolic extracts respectively. In contrast, methanolic extracts of both species inhibited growth of 100 % of the Gram-positive bacteria tested. None of the extracts displayed broad antifungal activity. Indeed, none of the extracts inhibited the growth of *A. niger* or *C. albicans*. Only *S. cerevisiae* growth was affected, and then only by the chloroform and hexane extracts. The methanolic, aqueous and ethyl acetate extracts of both Syzygium species were toxic in the *Artemia franciscana* bioassay, inducing significant mortality at <1000 μg/ml.
Discussion. The inhibitory bioactivity of *S. australe* against the bacterial panel validate Australian Aboriginal usage of *S. australe* leaves as antiseptic agents and confirms their medicinal potential, although care is needed in the uses of these extracts for these purposes due to their reported toxicity.

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Antimicrobial and anticancer activities of fruit extracts of the southern African medicinal plant *Kigelia africana* (sausage tree)

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Introduction. *Kigelia africana* (KA) is a large tree which bears large sausage shaped fruits. The fruit, leaves, bark and roots are used in a wide variety of southern African ethno-medicine systems as an anti-infective agent and in the treatment of a variety of conditions including rheumatism, cancer, dysentery and STD's. Despite its widespread usage, many of the therapeutic properties of this plant have not been rigorously studied and verified.
Methods. KA extracts were prepared and tested for their ability to inhibit the growth of a panel of bacteria, fungi and protozoa of medicinal importance. The ability to inhibit the proliferation of several cancer cell lines was also tested. Toxicity was assessed by the Artemia nauplii bioassay.
Results. All extracts displayed broad spectrum antimicrobial activity, each inhibiting the growth of 39% of the bacteria and 50% of the fungi tested. Strong inhibitory activity was detected with MIC values as low as 0.70 μg/ml against some bacteria, although most measured MIC's were generally at least an order of magnitude higher than this. All KA extracts were more effective against Gram-negative than Gram-positive bacteria, inhibiting 46 % and 20 % of each respectively. All extracts were also effective in inhibiting the gastro-intestinal protozoan parasite *Giardia duodenalis*. Similarly, all extracts blocked the proliferation of several cancer cell lines, with the ethyl acetate extract having the greatest efficacy. The methanolic and aqueous extracts had low toxicity in the *Artemia franciscana* bioassay. In contrast, the ethyl acetate extract proved to be completely nontoxic (LC50 > 1mg/ml).
Discussion. The inhibitory bioactivity against a range of microbes and their anti-proliferative activity against cancer cell lines indicate their potential in the discovery and development of new pharmaceuticals.

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Wound healing from the outback: novel wound healing therapeutics from native Australian plants

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Introduction: Chronic wounds are an area of major concern. As a result, pharmacological therapies have been developed to address treatment insufficiencies, however, the availability of drugs capable of promoting the wound repair process still remain limited. The wound healing ability of various herbal plants is well recognised amongst Indigenous Australians. Hence, based on traditional accounts, we evaluated the wound healing potential of two native Australian plants.
Methods: Active compounds were methanol and aqueous phase extracted from dried whole plant leaves. Primary keratinocyte (Kc) and fibroblast (Fib) cells (denoted as Kc269, Kc274, Kc275, Kc276 and Fib274) were subcultured onto 48-well plates and incubated (37°C, 5% CO_2) overnight with growth media. The growth media was discarded and replaced with fresh growth media plus various concentrations of either plant extract. Following the completion of the Almar Blue® assay (a measure of cellular metabolism); the CyQUANT® assay, which is a measure of cell proliferation based on total nucleic acid content, was performed. Anti-bacterial activity of the plant extracts was assessed using the Hinton-Muller method.
Results: The results generated from the AlmarBlue® and CYQUANT® assays indicated that extracts from both native plants at various time points (0, 24 and 48 hours) and concentrations (31.25 ng/L, 62.5 ng/L, and 125 ng/L) were significantly higher (n=3, p=0.03 for Kc269, p=0.04 for Kc274, p=0.02 for Fib274, p=0.04 for Kc275 and p=0.001 for Kc276) compared with the positive controls. In addition, the results demonstrate that neither plant extract demonstrated cytotoxic effects.
Conclusion**:** Both native plants contain bio-active compounds that increase cellular metabolic rates and total nucleic acid content. In addition, neither plant is cytotoxic. Future experiments will be assessing the anti-microbial properties of the native plant extracts.

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530

A targeted siRNA based screen to identify calcium transporters involved in the calcium-dependent regulation of ABCC3 gene expression in a model of breast cancer epithelial-mesenchymal transition (EMT)

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Introduction. Invasion and metastasis are hallmarks of cancer and are linked to resistance to anticancer therapies. Increased expression of members of the ATP-binding cassette (ABC) transporter superfamily is also implicated in resistance to chemotherapeutics, and has been linked to invasive behaviour. We have previously identified the Ca^{2+}-dependent upregulation of ABCC3 gene expression in a model of breast cancer epithelial-mesenchymal transition (EMT), a process involved in metastasis. The Ca^{2+} transporter(s) involved in the regulation of ABCC3 gene expression in this model of EMT are yet to be identified.
Aims. To identify the Ca^{2+} channels and/or pumps involved in the Ca^{2+}-dependent upregulation of ABCC3 transporter gene expression in a breast cancer model of epidermal growth factor (EGF)-induced EMT.
Methods. To assess the role of Ca^{2+} transporters in ABCC3 gene expression, an siRNA-based screen targeting 24 Ca^{2+} channels and pumps using Dharmacon On-TARGET*plus* SMARTpool siRNAs was performed. MDA-MB-468 basal-like breast cancer cells were transfected with each siRNA SMARTpool for 48 h. Following transfection, cells were serum starved (0.5% FBS) for 24 h before induction of EMT via treatment with EGF (50 ng/mL) for 48 h. Quantitative RT-PCR was used to assess changes in ABCC3 mRNA expression following siRNA and EGF treatment.
Results. siRNA mediated silencing of the Ca^{2+} permeable ion channel, TRPM7, a previously characterised partial regulator of EMT in this model, did not inhibit ABCC3 gene expression following 24 h EGF treatment. However, siRNA screening identified other Ca^{2+} transporters that may act as potential regulators of EGF-induced ABCC3 expression at 48 h, for further characterisation.
Discussion. Induction of EMT by EGF in MDA-MB-468 breast cancer cells results in the Ca^{2+}-dependent upregulation of ABCC3 transporter gene expression. Further studies are required to characterise the Ca^{2+} transporters involved in the regulation of ABCC3 expression in this model.

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Biased Signalling of Endogenous Opioids at the Mu Receptor

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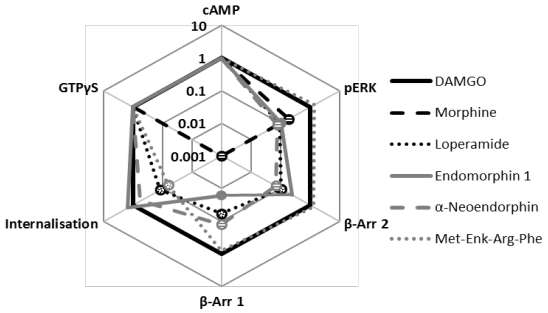

Introduction. The endogenous opioid system, with multiple ligands targeting the same receptor, may represent a natural example of functional selectivity or signalling bias, where different ligands binding to the same receptor generate different receptor conformations linked to distinct signalling pathways and a particular physiological response. Understanding these mechanisms can provide essential information on how opioid receptors regulate nociception and other physiological functions, and may lead to development of pain therapies that preserve potent analgesia while minimising side effects.
Aims. This study aims to elucidate cellular and molecular mechanisms elicited by endogenous opioids both in the central and enteric nervous systems, and to investigate the existence of functional selectivity in the endogenous opioid system.
Methods. Quantification of signalling bias of a range of endogenous opioids at the Mu opioid receptor (MOR) has been performed in a simple cellular model, to obtain unique ligand activity profiles or "fingerprints" for these ligands. The ability of each ligand to activate multiple signalling pathways was measured, and the bias between each pathway was quantified by the application of a novel analytical method based on the operational model of agonism.
Discussion. We found a number of endogenous opioids that exhibit signalling bias at the MOR. As signalling through GPCRs is highly dependent upon the cellular system, we are currently validating the ligand activity profiles of these endogenous opioids in primary dorsal root ganglia (DRG) and enteric neurons. This work will establish whether signalling bias by endogenous opiates exists in primary DRG and enteric neurons. Future work will examine the physiological outcome of biased signalling by endogenous opioids by using animal models for pain and gastrointestinal motility.

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Teaching 'old' polymyxins new tricks: Next generation lipopeptides targeting Gram-negative 'superbugs'
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Introduction. The clinically available antimicrobial peptides polymyxin B and colistin (Figure 1) are currently the last line of defense against many multidrug-resistant (MDR) gram-negative bacteria.
Aims. Our Hypothesis: incorporation of longer 'lipidic' side chains at position 6 or 7 of polymyxin B (Figure 1) will facilitate improved binding of the peptide to the modified Lipid A present in polymyxin-resistant strains, resulting in improved antibacterial activity against these strains. To this end we have synthesized a library polymyxin B analogues in which either the D-phenylalanine
residue at position 6 or the L-leucine residue at position 7 has been substituted with various natural and non-natural lipidic residues.

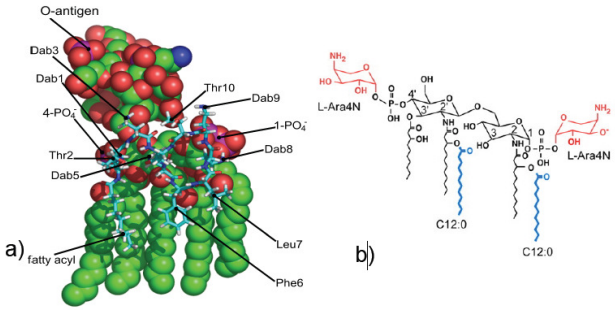

Figure 1. a). A molecular model of polymyxin B1 complexed to *E coli* LPS; b). Structure of Lipid A isolated from polymyxin-resistant *P. aeruginosa* showing the aminoarabinose modifications (in red) at the 4'-and 1'-phosphate and the fatty acyl modifications (in blue).

Methods. Synthesis of the protected linear precursor was carried out employing automated Fmoc solid-phase peptide synthesis on a CEM Liberty microwave synthesizer or Protein Technologies Prelude synthesizer, using Trityl-PS resin.
Results. Increasing the hydrophobicity of the resides at position 6 or 7 of polymyxin B significantly improved the MIC profile against polymyxin-resistant strains of *P. aeruginosa*. It also significantly improved the MIC profile against MDR-gram positive strains. The results obtained support the further investigation of these polymyxin analogues as potential therapeutics for targeting polymyxin-resistant gram-negative bacteria.
Velkov T et al (2010) J Med Chem 53:1898-1916.

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Pyrazolopyrimidine derivatives as translocator protein ligands with potential anti-glioblastoma properties
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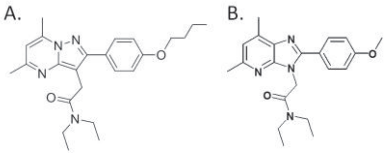
Introduction. The 18kDa translocator protein (TSPO) is upregulated in many cancers and neurodegenerative conditions. As such, it has become a target for the development of drug treatments for these conditions. Limited knowledge of the TSPO binding sites has hindered development of these drugs, however recent work suggests pyrazolopyrimidines may be a promising class of TSPO ligands (1).



Aims. We have synthesised a library of nine pyrazolopyrimidine derivatives aiming to examine the effects on TSPO affinity and efficacy of adjustments to the alkoxy substituent and hetrocyclic core.
Methods. Affinity was examined by competitive radioligand binding on HEK cells using [^{3}H]-PK11195, while a bromodeoxyuridine ELISA was used to examine anti-proliferative action on human T98G glioblastoma cells.
Results. Many of the pyrazolopyrimidines bound with high affinity to the TSPO, for example the methoxy derivative displayed a K_i of 11.3±0.94 nM. Functionally, however, only the butoxy analogue (Fig 1A) showed significant anti-proliferative effects on human glioblastoma T98G cells (64.5% inhibition at 100 µM; $p<0.001$). Shuffling the nitrogens around the heterocyclic core of the pyrazolopyrimidine to form an imidazopyridine (Fig 1B) decreased affinity (Ki = 30.03±2.91 nM) but created a functionally active ligand (10% inhibition of proliferation at 50 uM, $p<0.05$). Increasing or decreasing the number of nitrogens in the heterocyclic core (1, 2 or 4) created complex binding interactions, and decreasing affinity was observed with increasing nitrogen atoms.
Discussion. We have identified two anti-proliferative TSPO activators in a family of pyrazolopyrimidines, and found evidence that affinity for TSPO depends on the number of nitrogen atoms in the heterocyclic core of related compounds.
(1) Scarf AM, Ittner LM and Kassiou M (2009) J Med Chem 52:581-592

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Identification of calcium channels and pumps as therapeutic targets in breast cancer cells

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Introduction. Calcium channels and pumps are important regulators of many cellular functions. Studies show that aberrant expression of calcium transporters is a feature of some breast tumours. Calcium transporters that contribute to cancer cell proliferation may represent potential drug targets for the treatment of breast cancers.
Aims. To identify calcium channels and pumps involved in the regulation of breast cancer cell proliferation using pharmacological modulators.
Methods. Commercially available pharmacological activators or inhibitors (Sigma-Aldrich; Tocris) of calcium channels and pumps were used to screen for potential targets in MDA-MB-231 and SKBR-3 breast cancer cells. Cells were plated in antibiotic free media for 24 hours and incubated with compounds for 72 hours (4 days) before performing MTT assays (Promega) to determine the effect on viable cell number.
Results. From the 17 compounds tested, three compounds: N-(3-aminopropyl)-2-[(3-methylphenyl)methoxy]-N-(2-thienylmethyl)-benzamide hydrochloride (1:1) hydrate (AMTB), a transient receptor potential cation channel subfamily M, member 8 (TRPM8) inhibitor; mibefradil (a Ca_v3 blocker); and thapsigargin (a sarcoplasmic/endoplasmic reticulum calcium ATPase inhibitor) showed a pronounced effect, decreasing cell viability by greater than 70% in both MDA-MB-231 and SKBR-3 cell lines.
Discussion. Most research has focused on identifying calcium transporters as therapeutic targets through detection of calcium channels and pumps with altered expression in breast cancer cells. Our results show that pharmacological modulators may also be a useful, alternative method to identify novel targets in breast cancer. The transporters targeted by mibefradil and thapsigargin have already been extensively studied in breast cancer cells. However, the Ca^{2+} permeable TRPM8 ion channel requires further characterisation.

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Engaging and enhancing metabolism student learning capacity without compromising content or assessment standards

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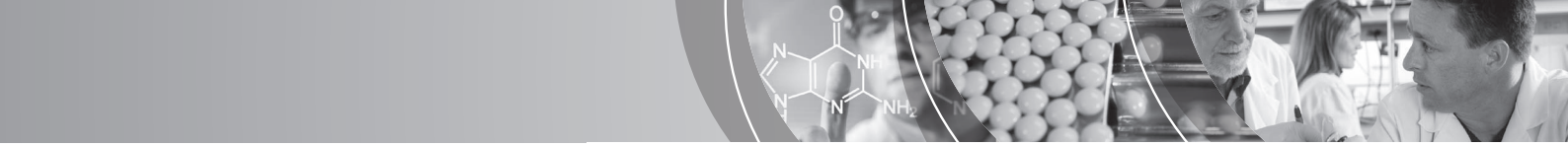
Introduction. Metabolism at Griffith University is a content heavy second year course with which students traditionally have struggled. It has traditionally suffered from an unacceptably high failure rate (>20%) in our school. Student perceptions of the difficulty of the course lead to disengagement prior to starting, with students not having acquired appropriate study techniques that would enhance their capacity to succeed.
Aims. This study aimed to develop a new teaching modality for the metabolism course and to monitor its effectiveness at increasing student outcomes and satisfaction, without compromising content or assessment standards.
Methods. Remodelling of the course in information sequence presentation and in presentation style occurred over several years. Content sequence changes ensured an understanding of key metabolic pathways followed by regulatory influences and disease states. The new format has a three week cycle that begins with traditional lectures but follows with workshops designed to cement core concepts and develop global cognitive comprehension. The cycle was designed to enable students to approach the study of this subject in a scaffolded manner that promotes engagement with materials and facilitates their overall capacity to learn in a deep manner.
Results. This strategy resulted in consistent reduction in the fail rate of approximately 10% (2008-2011) without compromising the standard of assessment. Students were guided to develop better self-efficacy and independent learning skills. Evidence of subject mastery is demonstrated by higher pass rates and also a shift to higher numbers of distinction and high distinction.
Discussion. Our scaffolded approach to teaching metabolism succeeded in significantly increasing student comprehension and satisfaction without compromising content or assessment standards.



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Prevalence of, and positive / negative issues surrounding, the use of e-learning tools

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Introduction. The use of e-learning tools in University teaching and learning is increasing, however little is known about the prevalence of, and the issues surrounding, the use of specific tools.
Aim. To investigate the prevalence of e-learning tools use as well as academic and student views on issues surrounding their use.
Methods. Academics and undergraduate students were surveyed at the University of Adelaide, Monash University and the University of Western Australia. Data collated included: demographics; frequency of specific tool use; perceived impact on teaching/learning effectiveness; and the positives/negatives of tool use.
Results. The survey was completed by 22 academics, with an average 13 yr teaching experience into 3 degree programs. The rank order of frequency of tool use was: audio lecture recordings > online quizzes/assignments > lecturer videos > wiki groups > discussion boards > blogs > podcasts. Positives aspects of these tools were that they allowed more flexible teaching and support for their use. However, the major drawbacks were the set-up time required, decreasing class attendance and technical reliability. The survey was completed by 276 students, who had an average of 4 semesters of full-time study. Although there were some differences between universities, generally the most frequently used tools were online quizzes/assignments, audio lecture recordings and lecturer videos, while blogs and podcasts were the least. The students also reported more effective and independent learning with tools as positives, while lack of interaction with lecturers, non-attendance at classes and technical reliability were the perceived negatives.
Discussion. Although the use of e-learning tools has been embraced across universities, and perceived by students and academics as allowing more flexible teaching and learning, the negative issues surrounding their use were substantial. The way in which these tools are utilised needs to address these obstacles to allow effective incorporation into our teaching programs.

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Development and implementation of a multi-disciplinary ethics broadening unit

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Introduction. The University of Western Australia (UWA) recently implemented a new course structure that includes broadening units chosen from outside the primary degree.
Aims. To describe the development and implementation of the multi-disciplinary ethics broadening unit entitled Social Responsibility in Action.
Methods. Five disparate disciplines across 4 faculties collaborated in the design of this 2nd year unit. The academic objective of Social Responsibility in Action was to increase student awareness and critical thinking skills related to a diverse range of ethical dilemmas. Common themes were developed to ensure coherence in learning material across disciplines. The student learning experience was examined in a survey (UWA Ethics Ref No. RA/4/1/5727).
Results. Philosophy first considers different approaches to ethical theory. Animal biology then considers animal ethics and welfare. Issues involving humans are addressed from anatomy and human biology as well as law perspectives. Pharmacology explores drug discovery and development in developed versus developing nations. A guest lecturer considers ethical dilemmas in the workplace. Tutorial-based assessments include discussion questions, quizzes, debates, briefing papers, public enquiries and group presentations. Students also select a discipline in which to complete a project. On a 7-point Likert scale, students Mostly Agreed or Completely Agreed that Social Responsibility in Action provided (i) a valuable educational experience (86%); (ii) an enjoyable learning experience (73%) and (iii) were better able to discuss the topics covered (90%). A student commented "*SCIE2100 has been able to provide me with valuable insights which I will not be exposed to within my area of studies. It has been able to trigger cognitive thought processes and it made me more aware of the ethical aspect of situations…*"
Discussion. Social Responsibility in Action was developed across UWA discipline silos. This unit seems to fulfil the students' need for awareness and critical thinking related to various ethical issues.

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The impact of a multi-disciplinary ethics unit on student ethical perceptions

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Introduction. A multi-disciplinary ethics unit Social Responsibility in Action is offered at The University of Western Australia (UWA) as either a broadening (Arts, Commerce or Design degrees) or an elective (Science degree) unit.
Aims. To examine whether student ethical perceptions alter following completion of Social Responsibility in Action.
Methods. Students enrolled in Social Responsibility in Action were asked to complete a survey (UWA Ethics Ref No. RA/4/1/5727) relating to their perspectives on a range of ethical issues at the start (pre) and end (post) of semester. The previously validated survey comprises two 10 item scales relating to ethical principles of idealism and relativism, with items rated on a 7 point Likert scale (Forsyth, 1980). Eighteen surveys were matched anonymously via the final four digits of students' mobile phone numbers. Survey responses were analysed as rating scores across each scale for each respondent. Responses were further rated as % agreement to each item. Two way repeated measures (ANOVA) was conducted on respondents' pre and post ratings and on the %agreement for each scale.
Results. Mean pre and post ratings on each scale were 5.3±0.7 and 5.1±0.8 for idealism and 4.5±0.9 and 4.5±0.8 for relativism. These ratings were significantly correlated; idealism ($r=0.73$, $p=0.0006$) and relativism ($r=0.79$, $p<0.0001$). A scatterplot of respondents mean ratings on both scales showed that most tended towards a situationist ethical stance, rejecting moral rules and advocating an individualistic analysis of each act in each situation. There was no significant difference between pre and post mean ratings, and no significant change in the ratings on either scale on completion of this unit.
Discussion. Most students adopted a situationist stance, recognising many different ways to look at morality (Forsyth, 1980). That there was no change in survey ratings may relate to the small number and heterogeneous nature of the students.

Forsyth DR (1980) J Pers Soc Psychol 39:175-184

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Why don't students attend lectures?

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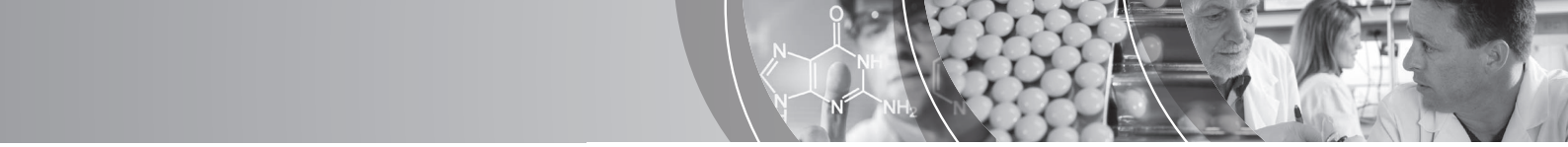
Introduction. Lectures form a common and efficient delivery mode in pharmacology education (Babey et al., 2010), although their effectiveness as a method of content and skills acquisition has been questioned in light of changes in pedagogical models and new technologies. Concerns about student attendance at lectures centre around the lack of engagement with course content as it is delivered.
Aims. To examine attendance rates and factors affecting attendance at pharmacology lectures at The University of Sydney.
Methods. Students enrolled in PCOL3022/3922 Neuropharmacology were surveyed (ethics approval #2012/865) at the end of the 13 week semester on factors affecting their lecture attendance (Davis et al., 2012). Data were analysed for mean and median responses as well as %agreement. Multiple linear regression (MLR) analysis was undertaken to determine predictors of self-reported attendance.
Results. Survey response rate was 40%, with 53% female, 72% in paid employment, 72% speaking English as a first language, and 98% enrolled full time. Respondents reported attending an average 1.3 out of 2 lectures per week, and average attendance over semester from head counts was 40%. Most students (up to 83%) disagreed that availability of lecture material online discouraged lecture attendance. 67% disagreed that university should move away from having lectures and 72% agreed that they would learn more if they attended lectures. The strongest influences affecting lecture attendance identified by students were timetable clashes and lack of sleep. MLR analysis further revealed early lecture time as significantly predicting lower attendance, supporting by the finding that students are less likely to attend early lectures.
Discussion. Findings from this study suggest students value lectures, however timetabling of lectures is an important consideration for student attendance and engagement.

Babey, A-M. et al. (2010) *ALTC Final Investigation Report*. http://www.olt.gov.au/resources/good-practice
Davis, E. et al. (2012). *Biochem. Mol. Biol. Educ*. doi 10.1002/bmb.20627.



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Operation of drug lists in Schools of Pharmacy

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Introduction. With more than 4000 medicines on the market, the number of medicines they are presented with to learn about understandably may overwhelm undergraduate medical students. Some medical schools have addressed this by adopting "medicines/drugs lists" which comprise of a limited number of medicines[1]. The composition of this list is arrived at through a number of ways. The same problem applies to undergraduate pharmacy students but it is not clear if pharmacy schools have addressed the problem in the same manner.
Aims. To ascertain which pharmacy schools in nine countries used "drug lists" in their curriculum.
Methods. Sixty eight schools of pharmacy in nine countries were sent a questionnaire enquiring in the first instance if they operated such a list and if so, what form it took, how the list was derived and whereabouts in the curriculum it was used. If the School did not operate such a list they were asked to give their reasons for not doing so. Schools were asked for a copy of their list.
Results. Forty three responses were received from schools, giving a 61% response rate. Eighteen of these schools operated a drug list. The majority of the schools (13) introduced the list in Year 1 of the course and all but one based the list on drug classes. Only one used disease basis for forming the list.
Discussion. The majority of pharmacy schools surveyed do not operate a drug list and despite 23 of them having a medical school in the same faculty, 19 of them did not know if that school operated a drug list. Only three pharmacy and medical schools in the same university operated the same list.
Conclusion: There is a great scope for further development of this concept and greater professional co-operation between medical and pharmacy schools staff.

1. Maxwell, S and Walley, T. Teaching safe and effective prescribing in UK medical schools: a core curriculum for tomorrow's doctors Br J Clin Pharmacol. 2003 June; 55(6): 496–503.

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Developing a blended approach to the teaching of neuromuscular pharmacology and toxinology

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Introduction. There is mounting evidence that 'blended' education programs, which combine meaningful integrated classroom teaching and e-learning components, are superior to traditional teaching in enhancing teaching efficiency and learning outcomes (Funke *et al*., 2013).
Aim. The current study aimed to develop a blended approach to the teaching of neuromuscular pharmacology and toxinology. The e-learning component of the module will be used as a self-directed learning tool in conjunction with traditional classroom teaching for medical, biomedical and other allied health students.
Method. A design framework for the neuromuscular pharmacology and toxinology blended module was constructed, based on learning tasks, which included animations, simulations and case studies, organized into pre-, in- and post-lecture activities. These activities demonstrate key skeletal neuromuscular transmission and toxinology concepts which are, where appropriate, illustrated by experimental data obtained from *in vitro* studies.
Results. Pre-lecture activities were developed and included animations introducing basic knowledge of skeletal neuromuscular transmission and neurotoxins, and assessments of these concepts. In-lecture activities consisted of learning activities based around envenoming case studies. Post-lecture activities contained more complex case studies of envenoming and neuromuscular disorders, and simulations illustrating more advanced concepts, such as interaction between skeletal muscle-targeting drugs.
Discussion. Pre-lecture learning tasks were aimed at ensuring a similar level of baseline knowledge for students prior to the lecture, where application of pre-existing knowledge and discussions of other aspects regarding envenoming could take place. Finally, post-lecture learning tasks were intended to enhance consolidation and further exploration of knowledge. The blended learning module contains educational resources that have been found to be beneficial towards student engagement and performance. Therefore, this approach is anticipated to have a positive impact on the teaching of neuromuscular pharmacology and toxinology to a range of students.

Funke K et al (2013) Langenbecks Arch Surg 398:335 – 340

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Assessment of pharmacists' knowledge and application of pharmacologic risk assessment tools in older people using a continuing professional development education method

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Introduction. The Drug Burden Index (DBI) is a pharmacologic risk assessment tool that measures an individual's total exposure to anticholinergic and sedative medications, and has been associated with impaired functional outcomes in older adults. There is a potential for pharmacists to use the DBI as a clinical tool to advise changes to exposure of these medications in older adults when conducting medication management reviews (MMRs). Education is fundamental to changing behaviours to improve professional practice and influence patient healthcare outcomes.
Aims. To educate pharmacists on pharmacologic risk assessment in older patients and to assess their knowledge using Continuing Professional Development (CPD) education.
Methods. The intervention was an educational article on issues such as polypharmacy, DBI and prescribing surrounding a fictional patient case, followed by four multiple-choice questions (MCQs) which, when answered, provided CPD credits for pharmacists. De-identified information on participants completing the CPD activity was obtained including age, gender, area and locality of practice, MMR accreditation status, and the answers to the four MCQs. Descriptive analyses were used to describe participant characteristics and performance in the MCQs.
Results. The MCQs were completed by 2,522 participants with the majority of participants female and a median participant age of 38 (IQR=27). Participants were mainly from New South Wales (31.8%), practising in community pharmacy (71.5%) and were not accredited to conduct MMRs (82.3%). The majority of participants were given full CPD credits for completing the exam (97.9%), however only 76.5% of participants received full marks. The question which required calculation of the DBI for a fictional patient was the lowest scored question.
Discussion. Our findings suggest that pharmacists have good knowledge of pharmacologic risk assessment tools. An electronic calculator may be required to facilitate use of the DBI as a clinical tool to optimise prescribing in older adults.

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Developing a clinical pharmacology (CP) training position in Perth, Western Australia (WA)

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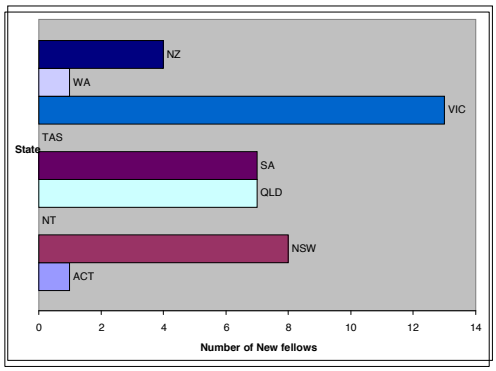
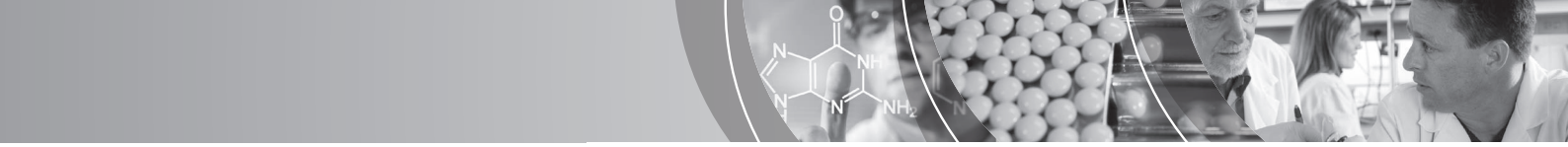
Introduction. Access to CP training for physician advance trainees in WA is constrained by the distance to eastern states. A local CP training program commenced as a combined training program with another RACP subspecialty training program based at Royal Perth Hospital (RPH) in 1999.
Aims. Review current CP training program in WA and careers of qualified fellows from WA program.
Methods. Literature review and survey with follow up face to face interview.
Results. There have been 4 trainees in the WA CP training program since its inception. 3 completed training and 1 candidate dropped out. 3 trainees combined CP with Geriatric medicine (geris) training and 1 trainee combined with general medicine (gen med). The first 3 trainees were CP+geris and the fourth trainee was CP+gen med. One of the CP-geris trainees did not complete combined training and only finished the geris component. The graph shows a comparison of the number of new fellows who graduated from CP programs around Australia and New Zealand since 2008 (RACP, 2013). 1 in WA out of 40 in last 5 years.



Discussion. The number of new physicians trained CP in WA is below that of other states and does not represent its potential based on pro rata population. The two clinical pharmacologist who are geriatricians work clinically in geriatric medicine and serve on CP committees such as CATAG, WATAG, PBAC and DUSC. The most recent graduate is the medical director of a research trials facility in WA. The current training program in WA is under review and a hybrid training program with a metropolitan wide focus is being considered.

RACP Education Office Communication (2013), Royal Australian College of Physicians, Macquarie Street, Sydney, NSW, Australia.



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Acrolein relaxes mouse isolated tracheal smooth muscle via a TRPA1-dependent mechanism

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Introduction. Activation of airway sensory C-fibres by the TRPV1 activator capsaicin causes relaxation of mouse isolated airway smooth muscle via the local release of neuropeptides (Taylor *et al*., 2012). Airway sensory C-fibres also express TRPA1 channels which have recently been identified as a key chemosensory receptor for acrolein, a toxic and highly prevalent component of smoke. However, it is not currently known whether acrolein-induced activation of TRPA1 produces relaxation of mouse isolated airway smooth muscle.
Aims. To examine the effects of acrolein on airway smooth muscle tone in mouse isolated trachea, and characterise the cellular and molecular mechanisms underpinning the effects of acrolein.
Methods. Isometric tension recording studies were conducted to characterise acrolein-induced relaxation of mouse isolated tracheal segments pre-contracted with carbachol. Use of selective antagonists and inhibitors permitted pharmacological characterisation of the molecular and cellular mechanisms underlying this relaxation response.
Results. Acrolein (1μM-100μM) induced dose-dependent relaxation responses in mouse isolated tracheal segments. For example, 30μM acrolein produced 67±12% relaxation in segments pre-contracted with 0.3μM carbachol (n=4). Importantly, these acrolein-induced relaxation responses were significantly inhibited by (1) the TRPA1 antagonists HC-030031 (20μM, 50% inhibition, n=3, $p<0.001$) and AP-18 (30μM, 92% inhibition, n=3, $p<0.01$), (2) an NK_1 receptor antagonist RP-67580 (20nM, 66% inhibition, n=6, $p<0.01$), and (3) the EP_2 receptor antagonist PF-04418948 (100nM, 65% inhibition, n=3, $p<0.001$). Acrolein-induced relaxation responses were completely abolished by the non-selective COX inhibitor indomethacin (5μM, n=4, $p<0.001$).
Discussion. In summary, acrolein induced a novel bronchodilator response in mouse airways. Pharmacologic studies indicate that acrolein-induced relaxation likely involves the activation of TRPA1 channels on sensory C-fibres. It is postulated that acrolein-activated sensory C-fibres release neuropeptides that stimulate NK_1 receptors on neighbouring epithelial cells. Finally, neuropeptide–activated epithelial cells release relaxant prostanoid products, such as PGE_2, which stimulate EP_2 receptors on airway smooth muscle to induce relaxation.

Taylor SJ et al (2012) *J Pharmacol Exp Ther* 340:377-385.

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Cytochrome P450 2A5 a mitochondrial bilirubin oxidase?

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Introduction. Bilirubin (BR), a recognized neurotoxin and antioxidant, is produced in mitochondria and microsomes. Excessive BR is eliminated through glucuronidation catalysed solely by microsomal uridine-diphosphate-glucuronosyltransferase 1A1 (UGT1A1), an enzyme that is not present in mitochondria[1]. In mitochondria BR is regulated by oxidizing enzyme that depends on mitochondrial electron transport chain[2].This enzyme has yet to be identified. We recently discovered the microsomal cytochrome P450 2A5 (CYP2A5) enzyme oxidises BR to biliverdin (BV)[3]. Because microsomal CYP2A5 is targeted to mitochondria, more so in response to oxidative stress[4], it is plausible that it can function as mitochondrial BR oxidase.
Aims. To explore mitochondrial CYP2A5 potential in oxidising BR.
Methods. Microsomes and mitochondrial fractions were isolated from liver homogenates of mice treated with sub-toxic dose of pyrazole. CYP2A5 protein levels and activity were assessed by Western immunoblotting and fluorometric method, respectively. BR oxidation was determined spectrometrically.
Results. CYP2A5 protein levels and activity in both organelles significantly increased in treated mice. UGT1A1 protein levels was not detected in mitochondria but remained unchanged in treated microsomes. BR oxidation in both organelles increased after treatment and BR affinity for mitochondrial CYP2A5 was as strong as microsomal enzyme.
Conclusion. Mitochondrial CYP2A5 can oxidise BR and its targeting to mitochondria during oxidative stress may facilitate BR regulation.

1. Radominska-Pandya et al. (2005) *Methods Enzymol,* 400:116-47.
2. Hansen et al. (1999) *Mol Genet Metabol,* 68:404-409
3. Abu-Bakar et al. (2011) *Toxicol Appl Pharmacol,* 257:14-22
4. Genter et al (2006) *Biochem Biophys Res Commun,* 342: 1375-1381

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Evaluation of *in vitro* assays as alternative strategies in predicting skin sensitisers

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Introduction. Allergic contact dermatitis (ACD) is a type IV hypersensitivity immune response which is mediated by T cells. Currently, the murine local lymph node assay (LLNA) is the 'gold standard' method for classifying potential skin sensitisers. However, increasing emphasis on the 3Rs principles of reduction, refinement and replacement has driven the need to reduce, if not replace the use of animals in assessing toxicological endpoints. Hence, the development of non-animal test methods that can reproduce the accuracy of the LLNA in identifying potential skin sensitisers, remains a vital challenge in many industries worldwide.
Aims. Comparative evaluation of the *in vitro* human cell line activation test (h-CLAT) and the direct peptide reactivity assay (DPRA) with the LLNA for identifying the skin sensitisation potential of five chemicals.
Methods. Five chemicals with varying skin sensitizing potential were evaluated using the h-CLAT (Ashikaga et al. 2006) and the DPRA (Natch et al. 2008). For the h-CLAT, monocytic leukaemia THP-1 cells were incubated with each of the test chemicals for 24 h. Following incubation, expression of surface molecules, CD54 and CD86 on THP-1 cells were monitored using flow cytometry. For the DPRA, synthetic heptapeptide Cor1-C420 was incubated with each of the test chemicals for 24 h. The total amount of unreacted heptapeptide was measured using LC-MSMS. The results obtained from both *in vitro* tests were compared with those from the LLNA.
Results. Our data show an accuracy of 60% for h-CLAT and 80% for DPRA with respect to published LLNA results for the five chemicals assessed.
Discussion. The h-CLAT and DPRA have potential for replacing the LLNA in identifying chemical sensitisers. Challenges that remain to be addressed include the evaluation of chemicals with low solubility or chemicals that may require bioactivation in the skin to form a skin sensitizing metabolite.

Ashikaga T et al (2006) Toxicol in vitro 20:767-773.
Natsch A et al (2008) Toxicol Sci 106:464-478.

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Regulation by Rapamycin and NrF2 of the Expression Bile Acid Transporters in Liver Cells

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Introduction: Rapamycin, a new class of immunosuppressive drug is considered one of the most potent cancer chemo preventive agents, has recently been employed as protective approaches to inhibit tumour regrowth. Reactive oxygen species (ROS) and increases in intracellular Ca^{2+} concentration mediate Ischemia Reperfision (IR) injury during liver surgery and transplantation. Removal of ROS by antioxidant enzymes is one of the strategies to reduce IR injury. Previous studies in our laboratory have shown that rapamycin can induce expression of two antioxidant enzymes, heme oxygenase-1 (HO-1) and peroxiredoxin 1 (Prx-1) in liver and in isolated rat hepatocytes and also lowers the bile acid recovery. It has been suggested that the mechanisms involve activation of the transcription factor NrF2.
Aims: The aim of this research is to investigate the mechanism by which rapamycin affects bile acid transporters in liver.
Methods: The H4IIE rat liver (CRL-1548) cell line was employed. Quantitative PCR (qPCR) was conducted using probe-based strategies, β-actin as reference RNA, a Rotor-Gene 3000 (Corbett) and the ΔΔCT method.
Results and discussions: In H4IIE cells rapamycin caused an inecrease in expression of Mrp-2 mRNA with 0.1 and 0.5uM causing increase of 100% and 150%, compared to untreated control respectively. Rapamycin also induced Mrp-2 mRNA expression about 200% and 50% in rat hepatocytes. Rapamycin at 0.1uM and 0.5uM caused a 150% and 125% increase in expression of Ntcp mRNA in H4IIE cells. But in rat hepatocytes rapamycin decreased Ntcp mRNA expression to 80% and 50% at 0.1uM and 0.5uM respectively. No mRNA encoding BSEP could be detected in H4IIE cells but detected in rat liver cells.
Conclusions: There are significant differences between H4IIE ("Cancer") cells and rat hepatocytes. Inhibition of Ntcp expression in rat hepatocytes may account for inhibition of bile flow recovery by rapamycin.

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Oxidative metabolism of rosiglitazone in the maternal and fetal sheep and human liver microsomes

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Introduction. The likelihood of fetal drug exposure is high; however, fetal drug metabolism capacity is limited and its contribution in drug elimination is not well understood.
Aim: To investigate oxidative metabolism of rosiglitazone in the feto-maternal compartment in sheep and human as a route of non-placental elimination of drugs.
Methods. Maternal and fetal sheep and adult human liver microsomes were used to study rosiglitazone metabolism as well as the contribution of CYP2C8 and CYP2C9 enzymes in this metabolism. A substrate depletion method was used and concentrations of rosiglitazone were measured by HPLC.
Results. Velocity of rosiglitazone (10μM) metabolism in microsomes of the fetus was lower than the mother (0.054 ± 0.018 vs 0.312 ± 0.032 μmol/nmol.P450/min, n=6, P<0.001). Maximum reaction velocity (Vmax) in the sheep and human liver microsomes were 1.386 ± 0.325, n=6 and 2.870 ± 0.993, n=5, μmol/nmol.P450/min, P>0.5, respectively. Microsomal P450 content in the sheep was higher than humans (0.810 ± 0.190 vs 0.188 ± 0.040 nmol/mg.protein, P<0.001). Inhibitors of CYP2C8 and CYP2C9 decreased rosiglitazone metabolism in the sheep (8.5% and 20.7%, respectively) and humans (7.3% and 16.7%, respectively) but not the fetus (<1%). The obtained velocity profile in the sheep and humans demonstrated atypical multi enzyme and atypical single enzyme kinetics, respectively.
Discussion. Low oxidative metabolism of rosiglitazone in the fetus indicates that fetal non-placental drug elimination may not be well developed. Therefore, hepatic elimination in the maternal sheep and placental elimination in the fetus possibly act as significant routes of rosiglitazone elimination. Similar key contributions of CYP2C8 and CYP2C9 enzymes in the metabolism of rosiglitazone in the sheep and human indicate that the sheep model may be successfully used in the study of metabolism. However, different P450 contents and enzyme kinetics emphasize that care needs to be taken in interpreting data from sheep to humans.

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The Putative transmembrane domain 6 of the human Organic anion transporting polypeptide 1A2 (OATP1A2) determines transporter function via substrate recognition and protein quality control

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Introduction. The human organic anion transporting polypeptides (OATPs) are a family of important membrane proteins that mediate the cellular influx of various anionic substances including clinically important drugs. The transmembrane domain 6 (TM6) is a distinctive consensus 'superfamily signature' shared by all currently known OATPs. However, little is known about the significance of this relatively conserved region in transporter function.
Aims. This study investigated the role of TM6 in determining transporter function of OATP1A2, a classic OATP member.
Methods. Alanine-scanning mutagenesis was adopted to introduce single amino acid mutation into the TM6 region of OATP1A2 (aa245-266). Transport uptake assay, biotinylation and immunoblotting were performed to assess the function and expression of OATP1A2 and its mutants in over-expressing HEK293 cells.
Results. A complete loss of estrone-3-sulfate (E3S, an OATP1A2 model substrate) uptake in the mutants constructed within the regions of aa245-248 and aa261-266 was observed, while those mutants within aa249-252 retained ~50% of function compared to that of wild type. Interestingly, the protein expressions of W245A and W246A remained unchanged in conjunction to maintaining their full functions in methotrexate influx. Our kinetic analysis then confirmed that these two residues are critical in E3S recognition. Protein expressions of mutants within aa247-248 and aa261-266 were significantly reduced on cell surface and in whole cell. Further experiments with the treatments of proteasomal or lysosomal inhibitors indicated that any single amino acid modification within these two regions would result in transporter protein misfolding, thus impairing protein insertion into cell membrane as to exert its function.
Discussion. Our study revealed the essential role of TM6 played in the substrate recognition and protein quality control of OATPs, which are novel information, contributing to elucidating the structure-function relationship of these transporters.

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Transcriptional up-regulation of human UDP-glucuronosyltransferase (UGT) 2B15 and 2B17 by tamoxifen and its active metabolite 4-hydroxyl-tamoxifen in breast cancer cells

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Introduction. Excessive estrogen-signalling is involved in breast carcinogenesis and promotes breast-cancer growth. The most potent estrogen, β-estradiol, can be synthesized from testosterone by aromatase in breast cancer tissues. Testosterone is mainly eliminated through glucuronidation by three UGTs (2B7, 2B15, and 2B17). *UGT2B15* and *2B17* are expressed in breast tumours, and hence their activities have potential impact on breast cancer growth. We recently reported that *UGT2B15* and *2B17* are upregulated by estrogens in breast cancer cells.
Aims. To study the regulation of *UGT2B15* and *2B17* by tamoxifen and 4-OH-tamoxifen in breast cancer cells.
Methods. Breast cancer MCF-7 and ZR-75 cells were treated with β-estradiol, tamoxifen, or 4-OH-tamoxifen with or without the estrogen receptor (ER) antagonist, ICI 182,780. Target mRNA levels were determined by quantitative real-time reverse transcriptase PCR. Knockdown of ER was achieved by siRNA silencing.
Results. Similar to the β-estradiol control, tamoxifen and 4-OH-tamoxifen at 1 μM significantly increased the mRNA levels of UGT2B15 and 2B17 in both MCF-7 and ZR75 cells. This increase in the levels of UGT2B15 and 2B17 mRNA was abrogated by either knockdown of the ER by siRNA silencing or the ER antagonist, ICI 182,780. Treating MCF-7 cells with 1 μM 4-OH-tamoxifen for 8 hours significantly elevated UGT2B15 and 2B17 mRNA levels. This effect was completely abolished by the transcription inhibitor, actinomycin D but not by the translation inhibitor, cycloheximide.
Discussion. Altogether, our results suggest that tamoxifen and 4-OH-tamoxifen stimulate the transcription of *UGT2B15* and *2B17* via the ER in breast cancer cells. This tamoxifen-induced UGT2B15 and 2B17 enzymatic activity would facilitate the removal of testosterone (the precursor of β-estradiol) and hence reduce β-estradiol synthesis in the tumour. This may represent a novel mechanism that may contribute to the antiestrogenic effects of tamoxifen, the cornerstone of endocrine therapy for estrogen receptor positive breast cancers.

Hu DG et al (2009) Mol Pharmacol 76:425-439

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Development of a novel HAMIN® topical local anaesthetic system for venepuncture

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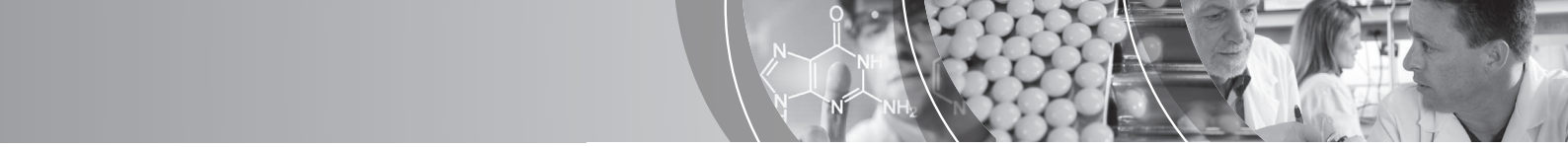
Introduction: Medical procedures are sometimes involved venepuncture. The pain caused by this minor procedure can leads to development of needle phobia which contributes the trivial problem not only in children but also in adults. Topical local anaesthetic agent was reported to be useful for pain relief causes by venepuncture.
Aim:The aim of this study was to develop a new formulation of topical anaesthetic cream using a novel self-emulsifying base called HAMIN®[Noordin et al, 2009]. HAMIN® is a natural base formulated from a mixture of Malaysian palm oil and palm kernel oil. Lidocaine was used as a local anaesthetic agent (LA).
Method: The cream was prepared using mixing by fusion technique which involved oil and water phase. Organoleptic test was carried on formulation to observe physical appearance of the cream whereas the viscosity and conductivity test were performed to identify the type of the cream and determine the flow behavior. The skin permeation test was conducted by using Franz type diffusion cell.
Results: Organoleptic test on the cream showed the appearance of milky white in color, odorless, non-greasy, washable, smooth and homogenous in texture. The conductivity test proved that the non-greasy properties of the cream because the cream was oil in water emulsion type. It was also identified to possess a pseudoplastic and thixotropic behavior from the viscosity analysis. Result of the permeation test indicated that the cumulative amount of lidocaine released from the cream and crosses the membrane was increased with time.
Discussion: It can be concluded that the new topical anaesthetic cream formulated using HAMIN® was an oil in water emulsion type with pseudoplastic and thixotropic behavior and suitable for local anaesthetic system due to the ability of lidocaine to cross the membrane in the diffusion test studied. The local anaesthetic behaviour of the cream will be studied in healthy human subjects.

Reference: Noordin M.I. et al (2009) Journal of Thermal Analysis and Calorimetry;95 (3):891-894.



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Differences in CYP1A2 mediated 7-ethoxyresorufin (ERES) *O*-deethylation between heterologous expression systems.

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Introduction: Human cytochrome P450 1A2 (CYP1A2) is an important hepatic enzyme responsible for the conversion of planar aromatic substrates to more polar metabolites, facilitating clearance from the body. CYP1A2 is critical in the metabolism of various clinically used drugs, and is responsible for the bioactivation of numerous procarcinogens. The elucidation of pharmacokinetic parameters from *in vitro* kinetic data is an important part of preclinical drug development. Thus, obtaining precise data is critical for accurate *in vitro*/*in vivo* extrapolation.
Aims: This study investigates kinetic differences in drug metabolism between recombinant human CYP1A2 enzyme generated from bacterial (*E. coli* cells), baculoviral (Sf9 cells), and mammalian (HEK293T cells) expression systems using the prototypic substrate, 7-ethoxyresorufin (ERES). Additionally, the study aims to determine whether inclusion of BSA in incubations (to sequester inhibitory fatty acids) impacts on the kinetic parameters derived for CYP1A2 mediated ERES *O*-deethylation.
Methods: Molecular methods were utilised for cloning and transformation of the pCW-17α CYP1A2 and pACYC-rOXR vectors into DH5α cells, and to isolate the co-expressed protein. PCR was used to create the pEF-IRES CYP1A2-V2A-OXR vector, which utilises the picornavirus V2A cleavage peptide. *In vitro* kinetics was measured by quantifying metabolite formation using HPLC.
Results: ERES *O*-deethylation by CYP1A2 exhibited Michaelis-Menten kinetics: bacterial (K_m 0.496 ± 0.08 μM; V_{max} 0.663 ± 0.09 pmol/min/pmol P450) and baculoviral (K_m 0.103 ± 0.01 μM; V_{max} 0.628 ± 0.02 pmol/min/pmol P450). The addition of 2% BSA resulted in an increase in K_m, affecting intrinsic clearance (CL_{int}).
Discussion: Differences in K_m values revealed that protein generated by alternative expression systems differ kinetically. A 5-fold increase in the CL_{int} was observed in baculoviral CYP1A2, relative to bacterial CYP1A2 ($p<0.05$). The inclusion of BSA in experiments increased K_m, decreasing CL_{int}. These data highlight the need for a standardised expression system for *in vitro*/*in vivo* extrapolation.

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Terbinafine in combination for the treatment of resistant or refractory mycoses: Investigating optimal dosing regimens using a physiologically-based pharmacokinetic model

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Introduction. Terbinafine is increasingly used in combination with other antifungal agents to treat resistant or refractory systemic mycoses due to evidence of synergistic *in vitro* antifungal activity. High doses are commonly used but very limited data are available on systemic exposure of terbinafine at high dose and no assessment of pharmacodynamic target attainment has been made.
Aims. Using a physiologically-based pharmacokinetic (PBPK) model for terbinafine, this study aimed to predict total and unbound terbinafine concentrations in plasma with a range of high dose regimens.
Methods. This study used a modified version of a previously reported PBPK model for terbinafine (Hosseini-Yeganeh *et al*, 2002). Clinically relevant dosing regimens, including terbinafine 500mg twice daily, 250mg three times daily, 250mg twice daily, 500mg once daily and 250mg once daily (standard dose) were investigated; simulations were performed with Micromath Scientist 3.0. Simulations from the PBPK model were used to calculate predicted pharmacokinetic and pharmacodynamic parameters for each terbinafine dosing regimen.
Results. Predicted terbinafine concentrations accumulated significantly during the first 28 days of treatment; AUC/minimum inhibitory concentration (AUC/MIC) ratios and *f*AUC/MIC (unbound AUC/MIC) ratios increased by 54–62% on day 7 of treatment, and by 80–92% on day 28 compared to day 1, depending on dose regimen. Of the regimens investigated, terbinafine 500mg twice daily provided the highest systemic exposure; on day 7 of treatment, the predicted AUC, peak and trough concentration were approximately 4-, 1.9- and 4.4-fold higher, respectively, than with a standard terbinafine dose regimen of 250mg once daily. There was good concordance between model predicted and observed terbinafine concentrations, indicating good predictive performance.
Discussion. This study provides the first report of predicted terbinafine exposure in plasma with high dose regimens used in clinical practice. Combination therapy with terbinafine is a promising strategy for the treatment of resistant or refractory mycoses.

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The effect of broccoli consumption on the activity of drug-metabolising enzymes in Europeans and South Asians: Study protocol

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Introduction. Of the extrinsic factors determining variability in response to medicines, less is known about the complex effects of diet. Of interest, cruciferous vegetables and their isothiocyanate constituents increase cytochrome P450 (CYP) 1A2 activity *in vivo* but inhibit it *in vitro*. There is also *in vitro* evidence that phenethyl isothiocyanate (PEITC) inhibits CYP1A2, CYP2D6, CYP2C19, CYP2C9 and CYP3A4 metabolic activity. Little is known about the interplay between the effects of diet and geographic ancestry on the activity of major drug-metabolising enzymes.

Aims. This study will investigate short- and medium-term effects of ingesting a broccoli-enriched diet on the activity of five CYP drug-metabolising enzymes in humans and establish if this effect varies between individuals of different ancestries.

Methods. This study is an open-label, sequential, cross-over dietary intervention trial utilising a 'cocktail' of CYP450-specific substrates to measure the activity of five CYPs with and after a broccoli intervention. 40 healthy, non-smoking male volunteers (aged 18-55 years) of European (n = 20) and South Asian (n = 20) ancestry will be recruited. On study days, participants will receive 100 mg caffeine (CYP1A2), 20 mg omeprazole (CYP2C19), 25 mg losartan (CYP2C9), 30 mg dextromethorphan (CYP2D6) and 2 mg midazolam (CYP3A4) in 5% dextrose solution. Venous blood samples will be collected at 0, 1, 2, 4 and 6 h post-administration. Parent drug and metabolites will be quantitated in plasma using a validated LC-MS/MS assay, and metabolite/parent AUC ratios used to estimate enzyme activity. PEITC concentrations in plasma will be determined by HPLC. Day 1 will involve baseline phenotyping of CYP activity. 200 g of fresh broccoli will be consumed with the cocktail on Day 2 and with lunch and dinner for six days before the last dose of the cocktail. Participants will be genotyped for alleles associated with increased/decreased CYP activity.

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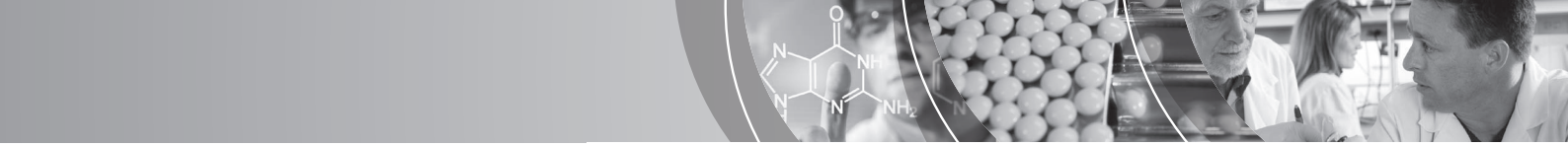
The role of CYP3A4 and CYP2C19 in the human liver biotransformation of the dipeptidyl boronic acid, bortezomib. Nuala Helsby[1], Betty Lee[1], Kathryn Burns[1], Malcolm Tingle[2]. [1]Molecular Medicine and Pathology, [2]Pharmacology and Clinical Pharmacology, University of Auckland, AUCKLAND, NZ.

Introduction: The anti-cancer drug bortezomib inhibits the 20S proteasome and disrupts the proteolytic degradation of cell signaling molecules resulting in cell death. There is extensive inter-individual variability in the pharmacokinetics of bortezomib (Reece et al 2011) possibly due to hepatic biotransformation by CYP3A4 and CYP2C19 to inactive deboronated diastereomeric carbolinamides (Uttamsingh et al 2005). Aim: To clarify the role of CYP2C19 relative to CYP3A4 in the inter-individual variation in the biotransformation of bortezomib. Methods: Bortezomib (0-100 μM) and cofactor (1 mM NADPH) were incubated (60 min) with either human liver microsomes (0.75 mg protein/ml) or CYP3A4/2C19 supersomes (10 pmol) in the absence or presence of inhibitors (CYP3A4: 2.5 μM ketoconazole; and CYP2C19: 10 μM omeprazole). Samples were extracted (0.4% formic acid in MeCN) and analysed by LC/MS. Results: Large variation in biotransformation was observed between livers (n=7). Both CYP3A4 and CYP2C19 supersomes catalysed the deboronation of bortezomib. In pooled microsomes, ketoconazole inhibited ($P<0.01$) bortezomib deboronation whereas omeprazole had no effect. Across individual livers (n=7), there was no correlation between immunoreactive CYP3A4 protein and deboronation of bortezomib ($R_s= -0.32$; $P>0.05$). In contrast, there was a negative correlation between bortezomib metabolism and CYP2C19 protein expression ($R_s= -0.964$; $P<0.001$). LC/MS analysis of biotransformation products indicated that CYP2C19 catalysed the formation of a putative tetrahedral hydroxy boronate. This metabolite was not observed with CYP3A4. In contrast, a putative imine amide was detected after incubation with CYP3A4 supersomes but was not formed by CYP2C19. Conclusion: Both CYP2C19 and CYP3A4 can catalyse the deboronation of bortezomib. However, CYP2C19 may produce an additional metabolite that inhibits catalysis by CYP3A4. The combined expression of both CYP3A4 and CYP2C19 may play a complex and unexpected role in the overall rate of biotransformation of bortezomib.

Reece et al 2011 Cancer Chemother Pharmacol. 67:57–67
Uttamsingh et al 2005 Drug Metab Dispos.33:1723-8



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***In vitro* assessment of metabolic drug-drug interactions potentially affecting olanzapine clearance.**

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Introduction. Olanzapine (OLZ) is an atypical antipsychotic prescribed to treat schizophrenia and related psychoses. Due to the overlapping pathology of mental illnesses (psychosis, depression and anxiety), OLZ is commonly co-prescribed with other psychotropic medications. OLZ is metabolised by various pathways and enzymes, the predominant routes being glucuronidation by UDP-glucuronosyltransferase (UGT) and *N*-demethylation by cytochrome P450 (CYP). As OLZ is a low hepatic clearance drug (CL_{PO} ~ 20L/hr), there is potential for co-prescribed medications to perpetrate metabolic drug-drug interactions that impair OLZ clearance.
Aims. Assess the capacity of commonly co-prescribed medications to inhibit the primary metabolic pathways involved in OLZ clearance.
Methods. The capacity of a panel of 11 psychotropic drugs (amitriptyline, caffeine, citalopram, fluoxetine, fluvoxamine, haloperidol, midazolam, nortriptyline, quetiapine, sertraline and zolpidem) to inhibit OLZ glucuronidation by rUGT1A4 and OLZ *N*-demethylation by rCYP1A2 was assessed *in vitro*. OLZ (350μM; rUGT1A4 or 250μM; rCYP1A2) and potential inhibitors (0, 1, 10, 100 or 500μM) were co-incubated in the presence of rUGT1A4 (0.1mg/incubation) or rCYP1A2 (4pmol/incubation). OLZ metabolite formation was quantified by UPLC-MS using conditions previously validated to assess the kinetics of OLZ metabolism by these enzymes.
Results. Fluoxetine (52% inhibition), midazolam (82%), sertraline (78%) and zolpidem (56%) inhibited OLZ glucuronidation by 50% or greater at an inhibitor concentration of 100μM. Similarly, fluvoxamine (96%), midazolam (67%) and sertraline (50%) inhibited OLZ *N*-demethylation by 50% or greater at an inhibitor concentration of 100μM.
Discussion. Screening experiments demonstrated that multiple commonly co-administered drugs have the potential to inhibit OLZ metabolism by either UGT1A4 or CYP1A2. Notably, sertraline caused potent inhibition of both the glucuronidation and *N*-demethylation pathways, and hence the potential for this drug to perpetrate clinically significant inhibition of OLZ clearance requires further characterisation.

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Imatinib PKPD relationship – Results from an ongoing study.

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Introduction. Imatinib is a tyrosine kinase inhibitor used in gastro-intestinal stromal tumours (GIST). The metabolic status of solid organ tumours (determined by PET scan) is an early indicator of therapeutic response. Trough concentrations (C_{24}) of imatinib may correlate with long-term response (time to disease progression [1]).
Aims. To determine if imatinib C_{24} correlates with an early measurement of metabolic response.
Methods. PK data was collected in 34 GIST patients on over the course of the study (3-42 months, 2-24 samples per patient). PET scan results were available in 24 patients. The PK data was analysed by NONMEM (Version 7.2). Correlation between early metabolic response (week 6-12) and imatinib C_{24} was made. The metabolic response was classified as either incomplete or complete.
Results. The pharmacokinetics of imatinib was best described by a one compartment model with first order absorption. The mean (CV%) of CL/F, V/F and Ka were, 10.0 L/h (31.0 %), 212 L (20.6 %) and 0.6 h^{-1} (fixed), respectively. Between occasion variability of CL/F was 14%. No covariates (e.g. age and body weight) significantly improved the fit of the model. Patients with an incomplete response (n=6) had a median (range) C_{24} of 0.8 mg/L (0.37-1.17 mg/L) versus 0.66 mg/L (0.31-1.08 mg/L) in patients with a complete response (n=18, P>0.05).
Discussion. Imatinib C_{24} did not correlate with the early metabolic response of the tumour. Tumour expression of human organic cation transporter 1 in chronic myeloid leukemia cell lines has been shown to determine imatinib response (2). We hypothesise that early response may not be related to plasma concentrations but to the tumour concentrations.

Demetri et al (2009). J Clin Oncol 27:3141-3147.
Wang et al (2008). Clin Pharmacol Ther 83:258-264.

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In vivo biodistribution of water-dispersible CdTe/CdS quantum dots after intravenous and subcutaneous injection

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Introduction. Quantum dots (QDs) are potentially useful in tumour diagnosis, as bio-indicators, and in drug delivery. Understanding QDs biodistribution after different administration routes is crucial for their safe and efficacious application.
Aims. To investigate the short- and long-term biodistribution of QDs after intravenous and subcutaneous injection routes.
Methods. Water dispersible cadmium telluride (CdTe) QDs (~3.5 nm) were synthesised in aqueous solution, purified, characterised and administered to mice at a dose of 0.02 nmol/g by intravenous or subcutaneous injection. Mice were sacrificed at various time points and blood and organs (liver, kidney, spleen) were collected. Cd concentrations in blood and organs were determined by ICP-MS after digestion with nitric acid and appropriate dilution.
Results. QDs showed bi-exponential plasma concentration-time profiles after intravenous injection. The profiles were well fitted by a two-compartment model. Plasma concentrations of QDs increased gradually after subcutaneous administration and reached a peak at 8 h after injection. QD levels in plasma plateaued after 24 h for both injection routes. QD concentrations in liver, spleen, and kidney were higher after intravenous injection than after subcutaneous injection until 24h. After 24 h, QD levels in kidney and liver were lower after intravenous injection than after subcutaneous injection. A time-dependent tissue redistribution into liver and kidney was observed after intravenous injection but not after the subcutaneous route. QDs were barely detectable in feces and urine after both intravenous and subcutaneous injection.
Discussion. Biodistribution of QDs is affected by administration route. Even up to 30 days, QD concentrations were still high in kidney and liver after both administration routes, which indicated QDs were retention in the body for a long time.

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The effect of the traditional Chinese Medicine Bu Zhong Yi Qi Tang on drug metabolising enzyme activity in healthy volunteers: Study protocol

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Introduction. Bu Zhong Yi Qi Tang (BZYQT) is a traditional Chinese Medicine consisting of 11 different herbal medicines that has been used for hundreds years for a variety of symptoms across South East Asia. Recent clinical research studies have shown that BZYQT may have beneficial effects for allergic rhinitis, dermatitis, fatigue and stress caused by cancer treatment. Therefore, BZYQT is one of the most commonly used Chinese herbal formulae as adjuvant therapy during chemotherapy or surgery in South East Asia. However, little is known about the potential herb-drug interaction of BZYQT with medicines used in treatment and management of people with cancer. This information is essential for the safe use of this medicine in the setting of cancer care.
Aims. This study will investigate the effect of co-administration of BZYQT on the activity of cytochrome P450 (CYP) drug metabolising enzymes, potential dose-dependent relationship of BZYQT on the inhibition or induction of CYP drug metabolising enzymes and the tolerability of BZYQT in healthy volunteers.
Methods. This is an open-label, sequential design, two dose levels, cross-over study in healthy volunteers (aged 18-50 years) who will be administrated a CYP phenotyping cocktail of 100 mg caffeine (CYP1A2), 25 mg losartan (CYP2C9), 20 mg omeprazole (CYP2C19), 30 mg dextromethorphan (CYP2D6), 2 mg midazolam (CYP3A4) before and after BZYQT treatment. Participants will be randomised (1:1) to receive one of two BZYQT dosing regimens (2.5 g or 4 g three times daily) from study day 2 to 8. Blood samples will be collected (over 6 h) after the CYP cocktail. Parent drugs and metabolites will be quantitated in plasma using a validated LC-MS/MS assay, and metabolite/parent AUC ratios used to estimate enzyme activity.

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Comparison of hepatic disposition of quantum dots and organic dyes in rat using multiphoton microscope

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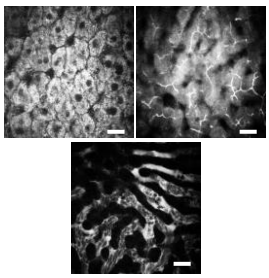
Introduction. Quantum dots (QDs) have several advantages over the organic dyes including broad excitation spectra, narrow emissions, high photostability, high quantum yield and resistance to photobleaching.

Aims. To compare the disposition of QDs in rat liver with cationic dye rhodamine 123 (RH123) and anionic dye fluorescein by directly imaging these substances using multiphoton microscope (MPM).



Methods. Water dispersible negatively charged cadmium telluride (CdTe) QDs (~ 2.1 nm) were synthesized in aqueous solution, purified, and characterized. QDs and organic dyes were injected into rat via jugular vein and were directly imaged in rat livers during anaesthesia after laporatomy using a DermaInspect MPM with a MaiTai femtosecond laser at 900 or 920 nm excitation. Fluorescence intensity of these substances was measured in time series.

Results. The figure shows the distribution of RH123, fluorescein and QDs in rat liver 30 min after intravenous injection. QDs distribute quickly and evenly in sinusoid without hepatocyte uptake and bile excretion. In contrast, fluorescein entered hepatocyte from sinusoid and then quickly concentrated into the bile, while RH123 was rapidly taken up from the sinusoids and concentrated in hepatocytes with slow excretion to the bile. Florescence intensity of QDs in the liver decreased much slower than that of organic dyes.

Discussion. Intravital imaging with MPM enables direct visualization of the space and time processes associated with QDs and organic dyes disposition in rat liver. QDs showed prolonged retention in liver sinusoid compared to organic dyes, which may be an advantage for *in vivo* imaging. However, as hepatic disposition is related to physicochemical properties of compounds, the size and surface properties may affect the hepatic disposition of quantum dots. Further studies should be done to evaluate these effects.

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Development and validation of an LC-MS/MS method for the quantification of the immunosuppressant, mycophenolic acid in human kidney transplant biopsies

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Introduction. Mycophenolic acid (MPA) has a low therapeutic index and large inter-individual pharmacokinetic variability, necessitating therapeutic drug monitoring (1.0 – 3.5 mg/L) to individualise dosage. There is an ongoing debate as to whether plasma MPA concentrations sufficiently predict kidney rejection or toxicity and whether concentrations within the graft tissue may better predict transplant outcomes.

Aim. To develop an LC-MS/MS method for the quantification of MPA in clinical biopsy samples from kidney transplant recipients taken as part of routine clinical procedures.

Methods. MPA was quantified retrospectively in 4 core needle kidney biopsies taken from 4 different kidney transplant recipients. MPA was also quantified in 2 kidney samples from rats administered MPA to assess tissue extraction reproducibility. Human kidney biopsies and rat kidneys were homogenised mechanically and underwent liquid-liquid extraction before analysis by LC-MS/MS. MPA-free human kidney tissue was used in calibrators and quality control samples. Analytes were detected using positive electrospray ionisation (MPA: m/z 321.1→ 207.3; internal standard N-phthaloyl-L-phenylalanine, PPA: m/z 296.2→250.2) .

Results. Calibration curves were linear from 0.6 – 20 ng/mL (R^2>0.99, n=10), precise, and accurate with coefficients of variation and biases of less than 15%. Extraction efficiencies for MPA and PPA were 97% and 86%, respectively, and matrix effects were minimal. In 4 kidney transplant recipients, graft MPA concentrations ranged from 1.3 – 7.7 ng/mg of tissue, and were within, or slightly above, the trough plasma C_0 therapeutic range for MPA.

Discussion. The LC-MS/MS method developed allowed the measurement of 1.2 ng MPA in kidney biopsies weighing as little as 0.1 mg. The ability of this method to utilise excess biopsy tissue allows MPA quantification to fit into current clinical protocols, hence facilitating an easier application of *in situ* immunosuppressant concentrations in the grafted organ as a potential guide to dose individualisation.

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Characterization of INS-1 832/3 cell line as a model system for studying GLP-1R regulation in diabetes

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Introduction. The glucagon-like peptide 1 receptor (GLP-1R) is a key physiological regulator of β-cell function and survival and currently targeted for treatment of type II diabetes. Type II diabetes is characterized by hyperglycemia, insulin resistance and β-cell dysfunction and long acting GLP-1R agonists, exendin-4 and liraglutide are approved therapeutics for this disease. The molecular mechanisms underlying GLP-1R physiology are highly complex and poorly understood, but $G_{\alpha s}$-mediated cAMP production as well as signaling via β-arrestins is implicated in augmentation of glucose sensitive insulin secretion (GSIS), enhanced β-cell proliferation and anti-apoptotic effects. Limitations of GLP-1 peptidomimetics has led to a continued interest in the search for small molecule GLP-1R agonists for therapeutics.
Aims. This study evaluates GLP-1 receptor responses to various peptide ligands with or without allosteric modulators in an endogenously expressing cell system. This forms a platform to assess the global cellular changes responsible for the positive profile of GLP-1R ligands in pancreatic β-cells.
Methods. Receptor activation has been assessed by measuring cAMP accumulation, GSIS, ERK1/2 phosphorylation, intracellular calcium mobilization, proliferation and apoptosis.
Results. INS-1 832/3 was identified as an appropriate model system when compared to other available cell lines. We show GLP-1R ligand dose-dependent effects on insulin secretion, ERK1/2 phosphorylation, proliferation and survival in the presence of high glucose. In low glucose, GLP-1R ligand dose-dependent cAMP accumulation was observed. Interestingly, all ligands behaved as inverse agonists in ERK phosphorylation assay at 5 min of stimulation in high glucose.
Discussion. These results elucidate the relative activities and interactions of GLP-1R ligands with small molecules and provide valuable insights into how GLP-1R signaling is modulated in presence of small molecule allosteric modulators. This may help in designing small molecule agonists with potential to either augument/mimic positive profile of GLP-1 peptides thus making them better therapeutics for the treatment of type II diabetes.

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Population pharmacokinetics of single-dose primaquine in Papua New Guinean children

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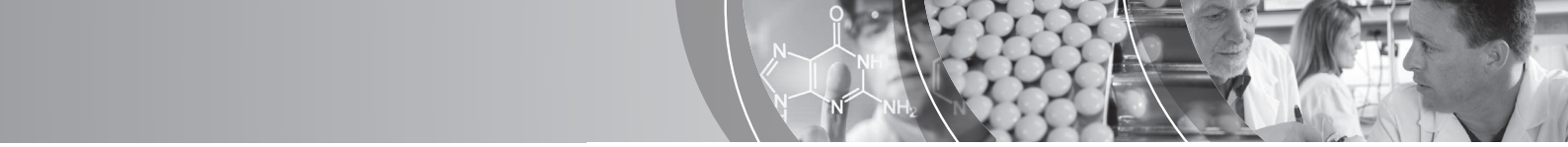
Introduction. Conventional 14-day primaquine (PMQ) radical cure of vivax malaria is associated with poor compliance and total dose, not therapy duration, determines efficacy
Aims. To develop a population pharmacokinetic model for PMQ and its metabolite carboxyprimaquine (CPMQ) in Papua New Guinean children, then to use the model to simulate conventional as well as multiple dose abbreviated high-dose regimens.
Methods. A preliminary pharmacokinetic study of two single doses (0.5 and 1.0 mg/kg) was conducted in 28 healthy glucose-6-phosphate dehydrogenase-normal Papua New Guinean children aged 5-12 years. Dosing was with food and directly observed, and venous blood samples were drawn over 168 h post-dose. Plasma concentrations of PMQ and CPMQ were determined by liquid-chromatography/mass spectrometry and population pharmacokinetic analysis was performed using non-liner mixed effects modelling with NONMEM.
Results. The mean PMQ central volume of distribution and clearance relative to bioavailability (200 L/70 kg and 24.6 L/h/70 kg) were within published ranges for adults. The median predicted maximal concentration (Cmax) for both PMQ and CPMQ after last dose of a 1.0 mg/kg 7-day PMQ regimen were approximately double those at the end of 14 days of 0.5 mg/kg daily, while a 1.0 mg/kg twice daily regimen resulted in a 2.38 and 3.33 times higher Cmax for PMQ and CPMQ, respectively. All predicted median Cmax concentrations were within ranges in adult high-dose studies that also showed acceptable safety and tolerability.
Discussion. The present pharmacokinetic data, the first for PMQ in children, show that further studies of abbreviated high-dose regimens are feasible in this age-group.



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Delivery of anti-inflammation peptides from polyurethane films

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Introduction. Implantation of medical devices is widely used for the management of numerous medical conditions including hearing loss, joint complications, heart valve failure and long-term drug delivery. However, immunological reactions to foreign materials can significantly inhibit the application and function of implanted devices. Advanced technology suggests that biocompatible material used in implanted devices may be a novel vehicle for the controlled release of therapeutics that could minimize or prevent adverse reactions to implantation.
Aims. The aim of our study is to understand how therapeutic agents might be incorporated and released from polymeric films used in medical devises. For this, we have investigated the impregnation of polyurethanes comprising different hard and soft segments with C5a receptor peptide antagonists. These peptide-loaded polyurethanes can be used as coatings on the surfaces of many implanted devices.
Methods. Peptide-loaded polyurethanes were prepared by solvent casting method. The amount of in vitro peptides (JPE1375 and PMX53) released was determined by liquid chromatography-mass spectrometry. The bioactivity of released peptides was evaluated by measuring intracellular Ca^{2+} mobilization in U937 cells.
Results. JPE1375, a linear peptide, is not stable at elevated temperatures and in many organic solvents commonly used to dissolve polymers. For both JPE1375 and PMX53, the peptides released from the polyurethanes maintained their bioactivity. The release of both peptides from Tecoflex can reach about 80% within 3 days. However, neither peptide was released to any significant extent from ElastonEon.
Discussion. These results suggest that the release kinetics of peptides is dependent on the interaction between peptides and hard or soft segments of the polyurethane, the thickness of films and the amount of drug loading. To prepare peptide-loaded polyurethanes, mild condition is required to maintain the peptides bioactivity. Release kinetics modification by blending nanoclays in polyurethanes and in vivo release studies are ongoing. These peptide-loaded polyurethanes may be useful for implantation application.

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Visualisation of endothelin-1–mediated contraction of rat airways and arteries and mouse airways *in situ* using lung slices

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Introduction. Intrapulmonary airways and arteries represent an important therapeutic target in many lung diseases, such as asthma and pulmonary hypertension. Endothelin-1 (Et-1) is implicated in these lung diseases as a potent constrictor of smooth muscle. The lung slice technique enables simultaneous visualization of intrapulmonary airway and artery reactivity *in situ*. However, whilst airway reactivity in this setting has been extensively characterized, assessment of vascular pharmacology within lung slices is relatively limited.
Aims. Our aim was to compare endothelin-1 reactivity between intrapulmonary arteries and airways in rat and mouse intrapulmonary airways.
Methods. Male Sprague-Dawley rats (8 weeks) or male Balb/C mice (6 – 9 weeks) were used for preparation of lung slices. Briefly, gelatin (4% in HBSS/HEPES) was injected into the right ventricle, followed by agarose (2% in HBSS/HEPES) into the trachea via cannula, with both gels allowed to set at 4°C, before slices (150 µm thickness) were cut using a vibratome. After overnight incubation at 37°C, changes in artery and airway areas within slices were imaged using phase-contrast microscopy during perfusion with Et-1 ± antagonists (bosentan, BQ123, BQ788), or dilator agents.
Results. Rat intrapulmonary arteries and rat and mouse airways (~200 – 300 µm diameter) all contracted in response to Et-1, with pEC_{50} values of 7.7±0.2, 7.9±0.4, and 8.5 ± 0.1, respectively. Greater maximum reduction in lumen area was seen in rat tissues (~70%) than mouse airways (~55%). Bosentan ~~(10 µM)~~ significantly antagonized Et-1-induced contraction in both arteries and airways in rat, and Et-1 contraction was mediated via $ET_B > ET_A$ receptors in mouse airways. The β_2-adrenoceptor agonist salbutamol relaxed intrapulmonary airways but not arteries.
Discussion. We have characterised contractile responses to Et-1 in rat and mouse lung slices. This technique can now be applied to disease models to explore the mechanisms underlying altered reactivity and for assessment of novel therapeutics for asthma and pulmonary hypertension.

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Bioactivity-guided fractionation of Australian native stingless bee (*Tetragonula carbonaria*) propolis extracts, based on *in vitro* free radical-scavenging and 5-lipoxygenase activities

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Introduction. Propolis, a resinous, plant-derived product of honeybees, exhibits anti-oxidant and anti-inflammatory properties (Búfalo et al, 2013). We found that a methanolic extract of Australian native stingless bee (*Tetragonula carbonaria*) propolis dose-dependently scavenged 2,2-diphenyl-1-picrylhydrazyl (DPPH) and inhibited 5-lipoxygenase activity *in vitro*. However, the active constituents of *T. carbonaria* propolis are yet to be elucidated.
Aim. To identify fractions within *T. carbonaria* propolis that scavenge DPPH and inhibit 5-lipoxygenase activity.
Methods. Propolis was collected from 40 *T. carbonaria* hives in South-East Queensland and extracted in 2:1 methanol:hexane. The crude methanolic extract was further separated into two hexane and one methanol-water sub-extract, which were evaporated to dryness and reconstituted in dimethyl sulfoxide. Sub-extracts (1-500 μg/mL) were tested for DPPH-scavenging activity (100 μmol/L; 30 min; 518 nm) and for inhibition of 5-lipoxygenase activity using colorimetry (Anthon et al, 2001). The methanol-water sub-extract was fractionated using preparative reversed-phase HPLC, and 11 fractions were collected, dried and re-tested for bioactivity using the assays described above.
Results. The polar, methanol-water sub-extract of *T. carbonaria* propolis was a more potent scavenger of DPPH than the hexane sub-extracts (EC_{50}=31.1±1.6 μg/mL; n=3; $P<0.05$), and dose-dependently inhibited 5-lipoxygenase activity (IC_{50}=42.8±4.6 μg/mL; n=3). Preparative fractions 1, 2 and 9 from the methanol-water sub-extract had greater DPPH-scavenging activity at 50 μg/mL than the other eight fractions (n=3; $P<0.05$). Fraction 1 also showed the greatest inhibition of 5-lipoxygenase activity at 100 μg/mL (n=5; $P<0.05$). Solvent controls were without effect.
Discussion. Chemical analyses of fractions 1, 2 and 9 are currently underway to identify the constituents of *T. carbonaria* propolis that possess free radical-scavenging properties and inhibit 5-lipoxygenase activity *in vitro*.

Anthon GE et al (2001) J Agric Food Chem 49:32-37
Búfalo MC et al (2013) J Ethnopharm 149:84-92

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Investigating the role of Nox2 oxidase and toll-like receptor 7 (TLR7) in influenza a virus (IAV)-induced reactive oxygen species (ROS) and cytokine expression in macrophages.

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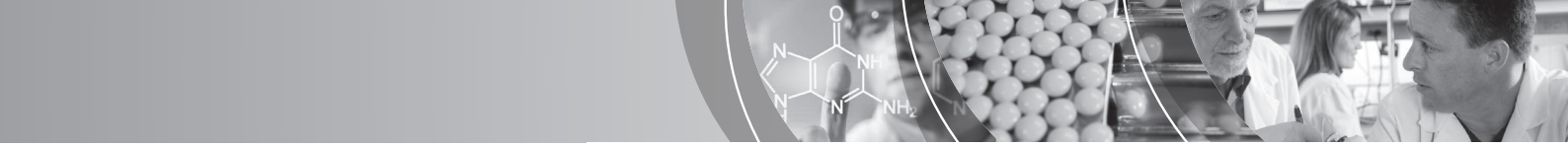
Introduction. IAV infects resident alveolar macrophages (AMs) of the respiratory tract, via dynamin and clathrin-mediated endocytosis, resulting in activation of endosomal TLR7 and ROS generation. Although Nox2 oxidase-derived ROS promote the lung injury to IAV, it is unknown in which subcellular compartment ROS production occurs and whether ROS influences antiviral cytokine production.
Aim. To establish whether IAV and TLR7 influence endosomal and extracellular ROS production and cytokine expression in macrophages.
Methods. RAW 264.7 macrophages and AMs were infected with HKx31 (H3N2) IAV strain (MOI 0.1-10), or treated with a TLR7 (imiquimod; 1-10μg/ml) agonist. Superoxide production was assessed using chemiluminescence and Oxyburst fluorescence microscopy. IAV, endosome and nuclear localization were examined by triple labelling immunocytochemistry and cytokine expression quantified by QPCR.
Results. X-31 virus internalized into early endosomes, which was blocked by the dynamin inhibitor dynasore (100μM), and caused an increase in endosomal superoxide production that was abolished by extracellular SOD and deletion of the Nox2 gene. X-31 infection significantly enhanced the phorbol dibutyrate (PDB)-induced oxidative burst and this 'priming' effect was abolished by a novel Ser346 HIV-tat containing peptide. Furthermore, imiquimod significantly increased ROS production, and similar to X-31, caused priming. Finally, AMs taken from Nox2-/- mice displayed a marked increase in IL-6, TNF-α, IFN-β and IL-1β expression compared to WT controls.
Discussion. This study demonstrates for the first time that IAV and TLR7 activation results in both an increase in endosomal and extracellular Nox2-dependent superoxide production, which suppress antiviral cytokine expression.



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Reduced number of mitochondria in NRF2 and NQO1 Knock-out mice

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Introduction. Nuclear factor (erythroid-derived 2)-like 2(Nrf2) is a transcription factor that regulates NAD(P)H quinone oxidoreductase 1 (NQO1) expression, which has been shown having important role in liver energy metabolism and regulatory functions in mitochondrial bioenergetics (Holmström et al, 2013). It is also central regulator of antioxidant and detoxification gene expression in response to electrophilic or oxidative stress (Nguyen et al, 2009). It has been shown that mice with genetic deletion of Nrf2 (Nrf2-KO) differ in stress and metabolic pathways on microarray analysis and have altered lipid metabolism (Chartoumpekis et al, 2013).
Aims. To understand if the absence of either NRF2 or NQO1 has an effect in liver metabolism through a change in mitochondrial number.
Methods. Livers from three wild type (WT) C57BL/6J, NQO1-KO and Nrf2-KO mice aged 6.5 mo were fixed for electron microscopy. Liver ultrastructure was assessed using transmission electron microscopy. Cytoplasmic area and number of mitochondria were manually calculated in ten hepatocytes for each liver using ImageJ sofware.
Results. The median and SD of mitochondria number in 100μm^2 areas in the three groups were respectively: WT, 33.89 ± 15.07; NQO1-KO, 30.4 ±11.7 and Nrf2-KO, 27.66 ± 7.5. The reduction of mitochondria in Nrf2-KO was highly significant ($P<0.0005$).
Conclusion. Nrf2 has an important role in liver metabolism by maintaining mitochondrial function and number.

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Respiratory syncytial virus induces glucocorticoid-insensitivity in airway epithelial cells

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Introduction: Respiratory syncytial virus (RSV) is a major cause of acute respiratory disease, especially in children. RSV infection responds inadequately to anti-inflammatory actions of glucocorticoids (Fernandes et al. 2013). Viral infection triggers expression and activity of cytokines, including transforming growth factor-β (TGF-β) (McCann et al. 207). We identified TGF-β as a mediator of glucocorticoid insensitivity in epithelium and airway smooth muscle (Salem Et al. 2012).
Aims: In the current study, we explore the contribution of TGF-β to RSV-induced glucocorticoid (GC) insensitivity.
Methods: Human airway epithelial cell line, BEAS-2B cells were inoculated with RSV at a multiplicity of infection of 0.1 for 1 hour, and incubated for 48 hour. GC-responsiveness was assessed by incubation the cells with budesonide (.01-100nM). Cells were transiently transfected with a Glucocorticoid Response Element (GRE)-SEAP reporter to examine the effect of RSV on budesonide-induced GRE activity. Endogenous glucocorticoid-responsive gene expression was measured by RT-qPCR.
Results: RSV infection profoundly inhibited budesonide-induced GRE activation. RSV infection also impaired budesonide-induced expression of GILZ (glucocorticoid-induced leucine zipper protein) and ENaCα (epithelial Na^+ channel alpha subunit), but enhanced MKP-1 (MAPK phosphatase-1) and IκBα (inhibitor protein of NFκB) expression in epithelial cells. Impairment of GC activity is prevented by the selective ALK5 (TGFβRI) inhibitor, SB431542, or the other two registered therapeutics with TGF-β modulating effects, pirfenidone and tranilast.
Discussion: RSV infection-induced glucocorticoid insensitivity is partially dependent on endogenous TGF-β receptor signaling. Establishing the effectiveness of tranilast and pirfenidone in viral infection supports the further investigation of the potential these drugs in prevention/treatment of asthma and COPD exacerbations.

Fernandes RM et al. (2013) Cochrane Database Syst Rev. doi: 10.1002/14651858
McCann KL et al. (2007) J Virol 81 (6): 2880-6
Salem S et al. (2012) Br J Pharmacol 166(7):2036-48