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FOREWORD

The **Welsh School of Pharmacy**, Cardiff University is the centre for pharmaceutical education and research in Wales. Its goals and activities are directed at improving the health, and alleviating the suffering from disease, of humankind. In 2009 the School is celebrating 90 years of pharmacy education, '**90 in 09**'. Research in the School currently encompasses aspects of all facets of the study of drugs, including: the discovery of new potential drug entities, using synthetic chemistry; formulation of drugs to achieve targeted responses; pharmacological studies of mechanisms of drug action; as well as researching elements of current medical and pharmaceutical practice. Some major internationally recognised research centres reside wholly or partially in the School. A number of research activities at the School involve collaborations with teams from overseas as well as from other institutions and organisations in the UK. Further information on the School's research programmes, along with contact details for academic staff, can be found at <http://www.cardiff.ac.uk/phrmy/research>.

If you are interested in undertaking postgraduate research please make informal enquiries with those staff members whose research interests you and/or obtain an application pack by e-mailing Terrett@cardiff.ac.uk.

The **Welsh School of Pharmacy** is highly rated for its undergraduate teaching provision. In its most recent visit by the Royal Pharmaceutical Society of Great Britain, the MPharm was re-accredited for a further period of five years. The School continues to attract large numbers of very well-qualified applicants for its MPharm degree from UK, EU and international students due to a number of factors, including the quality of the teaching and research.

All MPharm students at the **Welsh School of Pharmacy** are required to undertake a significant Masters level research project in the final year of the four year degree. In addition, fourth year MPharm students are also required to submit an abstract of their research and each one is included in this abstract booklet (except where publication and/or intellectual property issues, etc. restrict publication). Further, final year students are required to present their research findings when they are also assessed on their response to questions by academic staff. This year the Poster Day was held on April 23rd 2009.

The abstracts included in the 2009 **Welsh School of Pharmacy** MPharm Research Abstract booklet provide an indication of the ethos of the School's approach to research-led learning and teaching.

Within this publication the final year pharmacy student is the first named author and their name is underlined. An alphabetical list of authors, including those of supervising staff and collaborators, appears in the Index. For those who wish to cite an abstract the following may be used:

Authors' names. Title of abstract, *Welsh School of Pharmacy MPharm Research Abstracts 2009*, (ed. John DN) STS Publishing, Cardiff (2009) Page number. ISBN: 97809489173877.

My grateful thanks to my colleague Dr Keith Brain for his invaluable help with the publication of the abstract book. We hope that you find the abstracts are interesting and stimulating.

Dai John
July 2009

The following abstracts have been with-held as they have been or will be published elsewhere, and/or because of intellectual property or confidentiality issues and/or for other related issues.

Further synthesis and evaluation of aryloxy phosphoramidate b-2'-C-methylguanosine analogues against HCV

Kareem Adeniji and C McGuigan
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Efficacy of non-alcoholic hand rubs against intensive therapy unit isolates of *S. aureus*

Nishma Hirani and JY Maillard
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Prevalence and activity of prophages within *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients

Helen L Jones, CJ Cooper and JY Maillard
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Novel delivery of anti breast cancer agents

Lay Ming Lee and CM Heard
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Enhancing topical drug delivery

Gillian Susan Leslie Singka, NH Abu Samah, MH Zulfakar and CM Heard
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ProTides of gemcitabine as new potential anticancer agents

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Targeted inhibition of insulin-like growth factor type-1 receptor with a monoclonal antibody

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The synthesis of novel phosphoramidate derivatives of acyclovir as HIV reverse transcriptase inhibitors

David O Morgan and C McGuigan
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An evaluation of the use of the objective structured clinical examination (OSCE) in the Diploma in Clinical Pharmacy

Hannah E Simons and KL Hodson
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***In vitro* sublingual drug delivery**

Eleri Wallace and CM Heard
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Determination of the microbiological relevance of fluid samples

Noorsuriani Yusop, L Samuel,¹ R Burton² and J-Y Maillard
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Expression of endocytic proteins in native and amyloid precursor protein-transfected cell lines

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Alzheimer's disease (AD) is an irreversible neurodegenerative disorder characterised by progressive memory and cognitive decline. One neuropathological hallmark of AD is extracellular neuritic plaques containing beta amyloid, A β . The most critical pathology of AD - the production, processing and trafficking of A β - exploits the entire endocytic pathway. It is evident that endocytic changes involving both classical and non-classical endocytic pathways occur in AD¹. However, it is unclear how they occur, the extent of their involvement and when exactly they manifest themselves. This research aims to (i) establish, and detect any significant differences in, the expressions of seven endocytic proteins (flotillin, BACE 1, BACE 2, caveolin-1, caveolin-3, clathrin and PICALM) in two cell lines expressing native levels of amyloid precursor protein (APP, MOG-G-UVW and SH-SY5Y) and two cell lines transfected to overexpress APP (HEK293 and H4), and (ii) detect any differences in protein expression between the native and transfected cell lines. The project will determine the suitability of these cell lines as models for further research on endocytic pathology.

The HEK cells were transfected with Swedish mutation APP and the H4 with wild type APP695. They were cultured using standard techniques. Western blotting with commercially available antibodies was used, followed by densitometric analysis, ANOVA and post-hoc Bonferonni's tests.

Analysis revealed that, with the exception of caveolin-1 expression in SH-SY5Y cells, all other proteins were expressed at detectable levels by every cell line. The only significant differences in expression across the cell lines were for PICALM and caveolin-1. The MOG-G-UVW and HEK293 cells expressed more PICALM than the SH-SY5Y and H4 cells, while caveolin-1 expression was almost completely lacking in the SH-SY5Y cells. However, these differences were not correlated with APP overexpression.

To conclude, the cell lines expressed all the proteins in slightly variable amounts, and APP overexpression did not alter the expression of the endocytic proteins, making these cell lines suitable models for future studies. Future work could exploit primary neuronal cultures instead of immortalised cells, particularly of hippocampal or cortical origin, which are more relevant as they are implicated in AD.

1. Zhang, M. 2008. Endocytic mechanisms and drug discovery in neurodegenerative diseases. *Frontiers in Bioscience*. **13**: 6086-105.

The use of sports supplements by adult male gym users. Do community pharmacists have a role?

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The use of legal sports supplements has significantly increased over recent years.¹ The sports/energy drinks market alone is worth approximately one billion pounds per year in the UK.² At present community pharmacists do not generally have a role in sport/exercise related nutrition. Although the use of unlawful anabolic steroids has been reported,³ no published research studies were found relating to gym users' views of the potential role of pharmacists nor gym users use or knowledge of lawful supplements. It has been suggested that pharmacists may be well-placed to provide advice about sports supplements, but no published studies have supported this.² The present study aimed to investigate the use of sports supplements by adult male gym users and explore whether or not pharmacists currently, or could potentially, have a role in this area.

A multi-method design was used for this exploratory study. Following the granting of ethics committee approval semi-structured interviews were conducted to gather information about sports supplements and the issues relating to the general aims of this study.³ A purposive sample of nine participants (of differing ages and who trained for a variety of reasons) were recruited from a convenience sample of three privately owned gyms in Cardiff. A questionnaire was developed by reviewing available literature and using information obtained during the semi-structured interviews. The questionnaires were piloted using thirty gym members (ten from each of the three gyms). Structured face-to-face interviews using the questionnaire were conducted for the main study. Each gym was visited in the morning, afternoon and evening for two hours at a time for two weekdays and one hour on both a Saturday and Sunday at each of the three gyms (42 hours in total). This strategy was adopted to recruit gym users including the unemployed, students, those who worked 'office hours' and those who worked evenings and/or weekends.

In total, 106 structured interviews were conducted from 136 individuals approached (response rate of 77.9%). In total, 86.8% (n=92) of the participants used sports supplements. Protein powders (n=74), multivitamins (n=43) and energy/endurance drinks (n=41) were the most commonly used supplements. The majority of people used their supplements as recommended by the manufacturer and stated that they knew, or thought they knew, how their supplements worked. More than half of the participants (n=57, 53.7%) indicated disagreement with the statement "I would take advice about sports supplements from a community pharmacist". Almost half of the participants (n=51, 48.1%) indicated disagreement with the statement "Community pharmacists have a good knowledge of sports supplements". Approximately one fifth of gym users (n=25, 23.8%) indicated agreement with the statement "I would like to speak to a community pharmacist on how to get the best out of the sports supplements I am taking". The majority (n=104, 98.1%) of participants had never been to a community pharmacy to ask about the sports supplements they had used and approximately two-thirds (n=63, 59.4%) had never been to a community pharmacy to ask for advice about their own health.

This investigation confirmed that sports supplementation is widespread among adult male gym users in the sample studied although most of the participants have never been to a community pharmacy for supplementation advice. A very acceptable response rate was achieved and this is thought to be due in part to structured interviews being used rather than self-complete questionnaires. Further, the subject of the research was of interest to the participants with (86.8% using at least one supplement). The majority of participants indicated that community pharmacists did not have a good knowledge of sports supplements. However (if given an opportunity), some participants would speak to a community pharmacist about the sports supplements they were using. At present, community pharmacists do not play a role in the area of sports supplementation as they are not seen as a source of information or supply. However, as a first point of healthcare advice community pharmacists should have a basic knowledge and understanding of sports supplements. If pharmacists are to have a role then they and pharmacy students should be taught more about the use, safety and efficacy of sports supplements. A geographically wider study, using larger numbers of subjects, investigating the use of sports supplements by adult male gym users and the potential role of community pharmacists is warranted. Additionally, the views of community pharmacists regarding their role on sports supplements should be explored.

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2. Kayne SB. 2006. *Sport and Exercise Medicine for Pharmacists*. London: Pharmaceutical Press.
3. Monaghan, L (2001). *Bodybuilding, drugs and risks*. London: Routledge.
4. Gillham B. 2000. *The Research Interview*. London: Continuum. pp.9.

Cannabinoid mediated protection against oxidative injury in the isolated ileum

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Cannabinoids, both synthetic and endogenous, have been documented as displaying protective effects upon cells such as neurons following such damage as reperfusion injury and oxidative stress during inflammation. The majority of research into this area concerns the use of cannabinoids in neurodegenerative disease¹. However, there is limited research into its value in inflammatory diseases of the gastrointestinal tract despite some pathophysiological similarities of damage concerning nerves. Reactive oxygen species, a product of normal immune responses, cause damage to cells leading to changes in intracellular calcium concentrations and excitotoxicity and is thought that it is through these mechanisms cannabinoids protect². The aim of this study was to investigate if WIN 55,212-2, a non-selective cannabinoid receptor agonist, could protect against oxidative stress (via hydrogen peroxide (H₂O₂) exposure) in the isolated ileum.

Ileum was continually stimulated via electrical field stimulation (EFS) to display the contractile function of tissue throughout the experiments. Tissues were exposed to WIN 55,212-2 10⁻⁷ M alone or WIN 55,212-2 10⁻⁷ M in the presence of rimonabant 10⁻⁶ M. Following 10 minutes of incubation with WIN 55,212-2 the tissue was washed-out. Tissues were exposed to the drugs either before 60 seconds of H₂O₂ exposure or before and after and following which a recovery period for approximately 120 minutes after the oxidative insult. Return in EFS evoked contraction with time was measured as a percentage of the contraction measured before H₂O₂ exposure. Also measured was change in resting tension from the resting tension measured immediately following H₂O₂ wash-out. Paired tissue was used in each experimental set-up. Tissues exposed to ethanol were used as the control as it was the vehicle used for dissolving WIN 55,212-2 and rimonabant

Results showed that tissue exposed to a single dose of WIN 55,212-2 10⁻⁷M recovered significantly better than the control tissue and recovered much earlier. Tissue exposed to WIN 55,212-2 before and after H₂O₂ showed recovery but not statistically different from its control; similar results were found with the tissue once exposed to WIN 55,212-2 in the presence of rimonabant before H₂O₂ exposure. Tissue exposed to WIN 55,212-2 in the presence of rimonabant before and after H₂O₂ exposure showed recovery significantly better than that of the tissue exposed only to WIN 55,212-2. . The change in resting tension for all tissues showed a pattern of increasing to a peak at approximately 10 minutes after oxidative insult then decreased to its resting tension seen at H₂O₂ wash-out by 50 minutes. These results were found regardless of drug exposure.

All data showed some extent of recovery following exposure to WIN 55,212-2 indicating its ability to protect the ileum. Tissue exposed to WIN 55,212-2 in the presence of rimonabant appears to recover better. Rimonabant is a CB₁ antagonist and ought to attenuate the protection. The greater recovery of the tissue twice exposed to WIN 55,212-2 in the presence of rimonabant could explain that WIN 55,212-2 has both its protective effect and its pharmacological action of decreasing tissue EFS evoked tissue contraction³, introducing rimonabant inhibits this effect presenting the true ability for the tissue to recover and thus the real extent of recovery. The indifference of change in resting tension in tissues displaying protection indicates that calcium modulation may not be involved as calcium overload was still occurring. These findings indicates that perhaps WIN 55,212-2 does not protect through CB₁ receptors but through other proposed ideas such as functioning as an antioxidant. In conclusion, WIN 55,212-2 is able to protect against oxidative damage in the isolated ileum caused by H₂O₂. Some attempt was made to develop an understanding of the pathways, receptors and mechanisms involved however further research is required to develop these findings.

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2. RYAN, D., DRYSDALE, A. J., LAFOURCADE, C., PERTWEE, R. G. & PLATT, B. (2009) Cannabidiol targets mitochondria to regulate intracellular Ca²⁺ levels. *Journal of Neuroscience*, 29, 2053-2063.

3. LOPEZ-REDONDO, F., LEES, G. M. & PERTWEE, R. G. (1997) Effects of cannabinoid receptor ligands on electrophysiological properties of myenteric neurones of the guinea-pig ileum. *British Journal of Pharmacology*, 122, 330-334.

An exploratory study of schizophrenic patients' experiences and attitudes to clozapine

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Schizophrenic patients are often stigmatized by both the communities in which they live and by the healthcare professionals who treat them¹. Often their views on their drug therapy are left either unheard or unspoken. Clozapine, a last-resort drug treatment for treatment resistant schizophrenia (TRS) as recognized by NICE² (National Institute for Health and Clinical Excellence), is a notoriously problematic drug. Problems include a wide range of side-effects, strict monitoring guidelines and a complex dosing regime. The aim of this study was to ascertain, from patients themselves, their experiences of taking the atypical anti-psychotic drug clozapine and their attitude towards the drug itself.

The methodology of this study was quantitative and was based on validated rating scales found in relevant literature^{3, 4}. The method chosen to question patients was a structured interview. This was chosen because the provision of a rigid framework for patients to follow was important for gaining reliable data and preventing digression. The interview schedule was broken into three parts, sections A, B and C. In section A, patients selected side-effects they experience/experienced with clozapine from a series of show-cards. A measurement of severity and degree of bother was taken for each side-effect chosen using a five-point Likert scale. Section B focussed on clozapine-induced constipation. All patients were asked nine questions on lifestyle, features of constipation and constipation medications used, regardless of whether they selected the constipation show-card in section A. Finally, section C determined patients' attitudes towards compliance with clozapine from their responses to ten attitudinal statements. Patients were also asked with whom they discussed any side-effects experienced with clozapine. The inclusion criteria was that patients needed to be adults (over 18 years), capable of giving consent and currently being prescribed clozapine. Interviews were conducted face-to-face and patient responses noted. The interview schedule was piloted on four clozapine-prescribed in-patients located at Whitchurch Hospital. Patients for the study were recruited from two clozapine clinics in the Cardiff and Vale NHS Trust and were interviewed on site. SPSS 16.0 for Windows was used to input and analyse the data collected.

Thirty-six patients out of the thirty-eight approached consented to participate in the study. The most commonly selected side-effect of clozapine was hypersalivation (86%, n=31). Urinary retention was selected as both the most severe and the most bothersome side-effect according to mean scores. Constipation was the fourth most commonly selected side-effect (61%, n=22). Twenty-one percent (n=3) of those who did not select constipation as side-effect of clozapine were identified as being constipated according to a modified version of recognised diagnostic guidelines⁴. Most patients (83%, n=30) exhibited two or more features of constipation. The mean score for compliance attitude was 5.3 ± 4.4 (n=36) where max = 10 and min = -10. Two-thirds (24) of the thirty-six respondents admitted to discussing their side-effects with another person. The most popular group of people talked to were community psychiatric nurses (20%, n=8). The least popular were pharmacists (5% n=2).

The study revealed which side-effects are most trouble-some, thus providing healthcare professionals with relevant counselling points for prospective (and current) clozapine patients. This study also highlighted that constipation is an under-reported side-effect of clozapine. Patients' attitude towards compliance was seen to be very favourable. One suggestion is that the lengthy contact-time that clozapine patients share with healthcare professionals (due to the regular and mandatory clinic appointments) could be a factor for this phenomenon. Further studies using larger patient groups and more detailed diagnostic criteria of constipation would be beneficial in determining the extent of clozapine-induced constipation. Also, by implementing a test-retest feature into further studies the validity of future results will be stronger.

¹ Sartorius, N. 2008. Stigma and mental health. *The Lancet* **370**, pp. 810-811.

² NICE. 2009. *Schizophrenia Core interventions in the treatment and management of schizophrenia in primary and secondary care*. National Clinical Practice Guideline Number 82. London. NICE. Available at [WWW] <URL: <http://nice.org.uk/nicemedia/pdf/CG82FullGuideline.pdf>> [Accessed 01.05.09].

³ Day, J.C., Wood, G. et al. 1995. A Self-Rating Scale for Measuring Neuroleptic Side-Effects. *British Journal of Psychiatry* **166**, pp. 650-653.

⁴ The Rome foundation. 2007. *Rome III Diagnostic Questionnaires – Constipation module*. [WWW] <URL: <http://theromefoundation.org/pdfs/ConstMode.pdf>> [Accessed 01.05.09].

Vehicle effect on flux, and generation of prediction models

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A database of 480 drug/vehicle systems was collected from the literature using strict inclusion/exclusion criteria. Stepped single and multiple regression analyses were used in an attempt to produce an universal equation relating permeability coefficient (Kp) to variables: drug Mw, drug logP, vehicle logP, Hildebrand solubility parameters of drug and vehicle. The Potts & Guy approach¹ for Kp from aqueous vehicle uses Drug Mw and Drug logP as predictors. The regression had an R² value of 23.4%. Incorporation of the other variables improved this to 42.2%:

$$\text{Log(Kp)(cm/h)} = - 2.51 - 0.00506 \text{ Drug Mw} - 0.181 \text{ Drug LogP} \\ + 0.150 \text{ Hildebrand(v)-Hildebrand(d)} + 0.0962 \text{ Vehicle LogP}$$

suggesting that these variables have the potential for successful prediction as further data become available. The positive coefficient contradicts the assertion of Sloan et al.² that high permeability is associated with low difference in Hildebrand(v)-(d).

Regression analyses of the 'long-chain chemical enhancer' subgroup suggested that. Flux increases with molar volume (R²=27.4%) and enhancer logP (R²=41.6%). Further work should be conducted to determine which is the more important factor.

1. Potts, R.O. and Guy, R.H. 1992. Predicting skin permeability. *Pharmaceutical Research*. **9**(5), pp. 663–669.
2. Sloan, K.B. Koch, S.A.M. Siver, K.G. Flowers, F.P. 1986. Use of Solubility Parameters of Drug and Vehicle to Predict Flux Through Skin. *The Journal of Investigative Dermatology*. **87**(2), pp. 244-252.

Factors affecting the pharmacokinetics of fluoroquinolones in the lung

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Fluoroquinolones are a group of broad spectrum antibiotics stemmed from the quinolones. The first quinolone introduced was nalidixic acid. The quinolone pharmacophore was tested upon and it was found that the addition of a fluorine group on the C6 greatly improved the action.¹ Fluoroquinolones can be used to treat respiratory tract infections.² Their mode of action is via the inhibition of DNA gyrase which is selective for the bacterial version of the enzyme and inhibits RNA transcription and DNA replication.³ There has been recent study into the concentration of fluoroquinolones into different areas of the lung. The concentration observed in epithelial lining fluid and alveolar macrophages are now being recorded more regularly. The epithelial lining fluid coats the alveoli and is important for the action of antibiotics against extracellular pathogens and the concentration in the macrophages are important against intracellular pathogens.⁴ This project aims to collate data from different fluoroquinolones and investigate possible trends between the penetration into the lung and the compounds physical properties such as molecular radius and logP.

Papers were searched via electronic search engines such as Web of Science and Metalib. The papers were screened, and only used if; the studies were carried out in humans, there were concentrations measured in one or more of the epithelial lining fluid (ELF), alveolar macrophages (AM) and lung tissue (LT). The data was entered into Excel, and ratios were determined between plasma concentrations and concentrations in the different areas in the lung. LogP and molecular radius were established by drawing the structures in ChemDraw and the protein binding was taken from academic papers. From the protein binding the F_u was calculated as was C_u and ratios were made for C_u and the concentration in the lung. The physical data logP, molecular radius and F_u were plotted in Excel against the ratios made. In Excel the r^2 was calculated for the various correlations. To determine a significant correlation the value of r^2 was converted to r and using the table of critical values of Pearson's r , significance was established.

The results showed that there is no correlation between logP and the penetration of the fluoroquinolones present in the database. This was also the case for molecular radius as there was no significant relationship between the concentrations found and the shape of the molecule. Fraction unbound was shown to have some significant relationships, these included a significant correlation between concentration in the epithelial lining fluid and the lung tissue, but no in the alveolar macrophages. There were also significant correlations between the concentrations in the different areas with each other. C_u : ELF correlated with C_u : AM with all correlated with C_u : LT. The penetration into the alveolar macrophages were about 50 times higher than in the lung tissue and the concentration in the epithelial lining fluid was approximately 10 times higher than the lung tissue.

Molecular radius is a good indication of the shape of the molecule, which affects diffusivity. The reason there was no significant correlation between the molecular radius and the penetration of the compound could be because all the compounds have very similar shape and size, the molecular radius varied from 89.4 to 115. If there was more variation there could be a significant correlation seen. There was slightly more variation between logP 1.31 to 3.48, but no significant correlation. The correlations between the areas in the lung may be important. As the penetrations in the epithelial lining fluid, the alveolar macrophages and the lung tissue are all proportional the concentration could be estimated from one sample. As the sample of lung tissue is extracted from a biopsy which is very invasive, the concentrations could be estimated from a sample of the lining fluid instead, which also contains alveolar macrophages.

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2. Joint Formulary Committee. British National Formulary. 56th edition, British Medical Association and Royal Pharmaceutical Society of Great Britain, 2008. p. 320-323.

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4. Kiem, S., Schentag, J. J., Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. *Antimicrobial Agents and Chemotherapy*. (2008) 52.1; 24-36.

Assessment of sweetening agents used in pharmaceutical oral solutions

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It is now generally accepted that sucrose should not be used in paediatric formulations anymore due to the adverse effects that it may have on teeth. The objectives of this study were to determine potential sweetening agents to use in oral paediatric solutions as an alternative to syrup BP. Potential alternatives could be natural sweeteners such as glycerol and the polyols or artificial sweeteners such as saccharine. The taste of the preparation is important to patient compliance and therefore the solutions will be subjected to a taste panel. Osmolarity will be measured as the possible alternatives can act as osmotic laxatives¹. In addition viscosity, pH and antibacterial properties will also be determined. The data collected will then be assessed to achieve the ultimate objective of being able to provide a recommendation on a possible agent or mixture of sweetening agents to be used in future paediatric formulations. The data collected will hopefully provide information on possible alternatives to sucrose or provide opportunities to further explore possible alternatives.

Solutions were made of varying concentrations using syrup BP, glycerol, sorbitol and saccharine. Combinations of these sweetening agents were also made in an attempt to find an alternative to syrup BP. To gain information on the palatability of the solutions 5 volunteers tasted the solutions and completed questionnaire on sweetness, texture, location of taste and aftertaste. To determine whether additional preservatives would be required in the final formulation the antimicrobial activity of the sweetening agents was determined. Osmolarity of the solutions was measured to gain data on the possible laxative effect. To gain additional information on the solutions prepared which would help making the final formulation pH and viscosity were measured.

As expected the solutions with the highest concentration of sweetening agents achieved the highest mean sweetness scores. Glycerol was found to be sweeter than sorbitol, saccharine also helped make the solution much sweeter. An aftertaste was found in all solutions but was often a sweet taste which may be beneficial. All solutions showed adequate antimicrobial activity against *staphylococcus aureus* except for glycerol 10%w/v + sorbitol 10%w/v combination and the glycerol 20%w/v + sorbitol 10%w/v. Viscosities of the different solutions were similar and as the concentration increased so did the viscosity. The pH of the solutions were also similar as all solutions were weakly acidic. Glycerol was found to have a greater osmolarity than the other sweetening agents and the combination with a higher glycerol concentration also demonstrated a higher osmolarity.

After analysing the data the glycerol 10% w/v + sorbitol 10% w/v + saccharine 0.5mg/ml combination was deemed the best solution to replace syrup BP. Any of the combinations have the potential to replace syrup BP but as they failed the preservative efficacy test could not be recommended. The glycerol 10%w/v + sorbitol 20%w/v solution was excluded due to the higher concentration of sorbitol which would have a higher laxative effect. The glycerol 10% w/v + sorbitol 10% w/v + saccharine 0.5mg/ml has been chosen for its sweetness, preservative efficacy and minimum adverse effects on teeth. Other benefits of this solution is that due to a low concentration of glycerol and sorbitol the laxative effect should be lower especially as the glycerol will be more readily absorbed. While the solution does not have the highest viscosity none of the solutions have a very high viscosity and would all probably need to be modified in the final formulation. A bitter and sour aftertaste was reported for this combination but a sweet aftertaste was also reported more frequently and this may be beneficial. This is the solution that has the best properties but all of the mixtures have the potential to replace syrup BP as long as issues such as the antimicrobial activity can be proven. To back up the choice of solution we must look at a solution which has recently come to market. Ora-sweet SFTM is a solution available to flavour and sweeten extemporaneously compounded sugar free preparations. This formulation is made from 10% w/v sorbitol, 9% glycerol and 0.1% saccharine². Ora-sweet SFTM also has flavouring agents and xanthan gum to modify the viscosity³.

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Y-siting parenteral nutrition: Does current clinical practice jeopardise the stability of lipid emulsions administered to special care babies?

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Appropriate delivery of parenteral nutrition (PN) formulations, is an integral part of a successful neonatal care regimen and in age where there is capability to sustain life after as little as a 23-week gestation period, it has never been more important to identify and if possible, rectify those factors capable of jeopardising lipid emulsion instability, a required component of PN. For a number of years, Y-siting has been routinely used as the administration technique of choice however, investigations have since identified possible complications with its use¹. The objectives of the study were to identify the nutritional components of aqueous PN admixtures, as seen in current clinical practice, that have the potential to cause lipid emulsion instability and to then establish the clinical relevance of these findings through the use of administration set-ups as seen at ward level.

In order to fully achieve study objectives the study was designed to mimic clinical practice as closely as possible. PN volumes were manipulated using syringes under aseptic conditions and concentration of nutritional components calculated from the "European Society of Paediatric Gastroenterology, Hepatology and Nutrition" and the "European Society for Clinical Nutrition and Metabolism" (ESPGHAN-ESPEN) Guidelines². Investigations were carried out in two principal stages: Stage 1 involved the use of admixtures in glass bottles to identify which nutritional components posed the greatest risk to lipid emulsion stability; Stage 2 then involved running those admixtures through a Y-site simulation setup. Three principal particle size analysis techniques were used in order to identify the presence of any instability: Macroscopic, Microscopic and Malvern Mastersizer™ analysis (Stage 1 only). Samples were tested every 0-, 2- and 4 hours and in Stage 1 admixtures were placed in a waterbath set at 37°C over this time period.

With macroscopic, microscopic and Malvern Mastersizer™ analysis, admixtures with high amino acid concentrations were found to be more unstable than those with high glucose concentrations. Malvern Mastersizer™ assessment recording a % volume of globules > 4.3µm of 1.01% vs. 0.12%. Calcium proved most destabilising of all lone ions tested, destabilisation peaking at a concentration of 7 mmol.L⁻¹, with globules 10 µm in diameter present from 0 hours. With calcium concentrations =8 mmol.L⁻¹ macroscopic, microscopic and Malvern Mastersizer™ assessments saw a reduction in the amount of instability observed. In combination calcium 7mmol.L⁻¹, magnesium and Peditrace® caused a great deal of macroscopic and microscopic instability at 2-hours however, at 4-hours although there was still macroscopic instability present there was little microscopic instability most likely due to sampling variability. Stage 2 of testing using the Y-site simulation did not corroborate these findings, no macroscopic changes were observed and only a small number of globules between 5 and 15 µm were seen after 4-hours.

It has long been thought that amino acids provide a stabilising effect to PN admixtures³ however previous studies have shown that glucose does in fact provide a stabilising effect and it has been hypothesised that glucose is capable of reducing the hydration force between PN droplets⁴. It has also been hypothesised that glucose causes the slowing down of Brownian motion allowing for fewer interactions to occur between droplets and hence increase stability¹. It is not possible to say whether or not calcium has a greater destabilising effect on lipid emulsion as greater concentrations of calcium were added to admixtures as according to ESPGHAN-ESPEN Guidelines. At concentrations of calcium =8 mmol.L⁻¹ it is most likely that charge reversal was occurring due to changes in the electrostatic forces that stabilise lipid emulsion droplets. In the presence of cations these forces become weakened causing instability however, in the presence of more cation than is needed to neutralise the charge on lipid droplets lipid droplets become stable once again as all droplets acquire the opposite charge and repulsive forces dominate⁴. It is clear to see that at concentrations of PN admixtures as seen in clinical practice have the ability to cause lipid emulsion instability however, it is impossible to be certain whether or not this has any bearing on clinical practice due to study limitations, such as the lack of an incubator and time restrictions.

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A quantitative study to explore the public's perception of the roles of the pharmacist beyond dispensing

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Traditionally, the pharmacist profession has been associated with the safe, effective and accurate dispensing of prescription medication and devices. The government has since recognised that professional responsibilities must expand to improve patient care and well being, with the publication of the Crown Report 1999¹, which contains proposals for legislative change in the structure of the pharmacy profession to benefit healthcare. Only a limited amount of data exists regarding specifically the "general public's perception" of pharmacist roles. Therefore, this study aimed to explore the public's current views of the role of the pharmacist beyond their traditional dispensing role.

Quantitative research using a self-completion postal questionnaire was considered an efficient method of data collection, as it focused on the opinions and experiences of services from the perspective of the population surveyed². Wards of Cardiff were researched to locate an area which contained the highest density of demographics listed of the average pharmacy user. Profiles of the "average pharmacy clientele" who use the pharmacy more frequently, from previous studies, have included: mostly women, of lower socio-economic groups, who are middle aged and class their health as "fair"^{3,4}. Pilot research was conducted to ensure the validity and reliability of the questionnaire prior to its use on the final sample². Convenience sampling was used to recruit 25 participants, who had the same demographic characteristics of the subjects the research was aimed towards. A sample size of 350 participants were randomly chosen from the Llanrumney area of Cardiff. This allowed every person on the register an equal chance of being selected. Questions were mostly closed i.e. multiple choice tick boxes and Likert scales used as they would quantify data, were easier and quicker¹ for participants to carry out and therefore would increase response rate.

An overall response rate of 37.4% (n=131) was achieved. Questions focussed on a number of areas of the pharmacist role including: purchase of medicines, advice, prescribing, pharmacists' conducting face to face chats with patients about the use of their medication, pharmacist monitoring and screening and pharmacist access to patient medical records. Respondents generally agreed that antibiotics, the contraceptive pill, asthma relievers, anti-bacterial eye drops, anti-migraine medication and the flu jab should be available for purchase. Fifty seven percent (n=60) of respondents, for example, agreed with the availability of the contraceptive pill for purchase. For queries regarding side effects, 65% (n=78) of respondents chose the doctor as their preferred source of information, however, when the query related to controlling headache symptoms, smoking cessation and travel medication advice, the majority of respondents preferred the pharmacist. The majority of respondents generally agreed with pharmacist should be able to prescribe certain medication, where 60.5% (n=72) were in favour of pharmacists prescribing antibiotics for a chest infection. Sixty nine percent (n=83) of respondents agreed that pharmacists should be allowed to conduct face to face chats with patients about their medication use. Forty nine percent (n=60) of respondents disagreed with pharmacist access to patient medical records. When comparing age categories with participants preferred source of advice for smoking cessation and travel medication advice results suggested that there is a changing perception of the pharmacist's role over the generations. The older generation indicated they would still prefer to use the doctor for more 'serious' queries and for diagnostic and screening purposes in comparison with all age categories below 66 years.

There is support for the roles of the community pharmacist beyond dispensing. Results emphasised the need to increase the public's confidence, awareness and expectations in pharmacists' abilities and benefits to patient care, especially with regard to accessing full patient medical records. The pharmaceutical profession has come a long way over the last ten years. It is crucial, however, for community pharmacists to have the support of the general public for the profession to continue to develop and thereby improve healthcare and to continue to progress.

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Design, molecular modelling and synthesis of novel methylene blue analogues as antimalarial agents

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Malaria is accountable for almost one million deaths each year ¹ and resistance has emerged towards the majority of antimalarial agents currently used. A number of research incentives have recently been established to promote research into antimalarial compounds with novel mechanisms of action. Methylene blue is a glutathione reductase inhibitor which exposes *Plasmodium Falciparum* to high levels of oxidative stress. It selectively inhibits *Plasmodium Falciparum* glutathione reductase over human glutathione reductase. Methylene blue was the first synthetic antimalarial compound; its popularity was surpassed by the discovery of chloroquine. The decline in methylene blue use has hindered the development of resistance towards it. Methylene blue is an efficacious antimalarial but side effects include blue discolouration of bodily fluids due to its sulphur content and monoamine oxidase A inhibition. The aims of this study included *in silico* design and synthesis of dye-free compounds analogous to methylene blue.

Interactions between methylene blue and its target *Plasmodium Falciparum* glutathione reductase were studied ² and a basic structure was designed which would be incorporated into all analogues to reduce discolouration of bodily fluids. A database of 231 analogues was created and analogues were then docked within the target ³ and computational output variables were examined. Interactions between methylene blue and monoamine oxidase A were studied and methylene blue was also docked within monoamine oxidase A. Synthesis of the final analogues was then attempted. These synthetic steps differed from conventional synthetic procedures as sulphur was not incorporated into the analogues designed. Synthetic steps involving nucleophilic aromatic substitution via Ullman coupling, esterification and reduction were successful. The final cyclization step was attempted using conditions for Friedel-Crafts reaction which unfortunately was not completed.

Through molecular modelling studies the key interactions between methylene blue and amino acid residues of the tri-cyclic binding site of *Plasmodium Falciparum* glutathione reductase were determined. Based on these observations structural characteristics for non-competitive inhibition of *Plasmodium Falciparum* glutathione reductase were summarised. The final database contained 7 analogues which appeared to interact successfully with *Plasmodium Falciparum* glutathione reductase. The final 7 analogues were then examined to reveal similarities and trends between analogues. Similarities and differences between binding sites of monoamine oxidase A and *Plasmodium Falciparum* glutathione reductase have been highlighted and the final 7 analogues docked outside the predicted binding site; displaying selectivity for *Plasmodium Falciparum* glutathione reductase. Modification suggestions to minimise inhibition of monoamine oxidase A have also been made. In synthetic research, all essential synthetic steps were performed successfully except the final reaction which was attempted but not completed due to time limitations.

This study encompassing both design and synthetic aspects of novel antimalarial compounds was a success. Through molecular modelling studies a structural nucleus has been designed which allows analogues to interact successfully with *Plasmodium Falciparum* glutathione reductase. The final analogues designed share many similarities which each other and future study based on these molecules can provide further information on desirable characteristics for successful inhibition of *Plasmodium Falciparum* glutathione reductase. Although full synthesis of methylene blue analogues could not be completed the majority of synthetic procedures were successful; many synthetic challenges were conquered in this study. However with further work the synthesis of a sulphur free methylene blue analogue is achievable based on the synthetic knowledge gained in this study. It is also possible to incorporate the findings based on monoamine oxidase A to future study in an attempt to selectively target *Plasmodium Falciparum* glutathione reductase over monoamine oxidase A.

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Investigating the education and training needs of pharmacists in Wales

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Continuing professional development (CPD) is an ongoing learning process for pharmacists to maintain their knowledge, skills and competences. In order to fulfil their CPD needs, pharmacists may employ a range of formal and informal training activities. These training activities may provide from a professional organisations, University, employers and others. Little research has been conducted in exploring CPD engagement for pharmacist who qualified between 5 and 25 years. This survey aims to identify the learning activities that this sub-population of pharmacists in Wales, are currently using to support their CPD and also to understand their CPD support in future professional development needs.

A mailed-out questionnaire and accompanying cover letter were sent to 2044 RPSGB registered pharmacists across Wales. The questionnaire consisted five parts aim to determine pharmacists' CPD training activities and training providers used, motivating factors for CPD and future CPD supports needed. Closed and open ended questions were used in the questionnaire. SPSS version 16.0 was used to collate and analyse data. Microsoft Excel was used to generate figures.

In total, 842 questionnaires were returned and 371 questionnaires which returned from pharmacists who registered between 5 and 25 years were analysed. The response rate of this study is 41%. Most respondents were female (63.6%) and working full time (63.3%). The largest group of respondents worked in the community (46.9%), followed by hospital (20.8%), primary care (4.9%) and those working in other sectors (2.7%) The top three education and training providers that respondents have used in the past 12 months were WCPPE, Welsh Centre for Pharmacy Professional Education (84.1%), employer (53.1%) and the Welsh Medicines Resource Centre, WeMeReC (44.5%). Respondents the preferred independent learning and reading journals (online or paper) was the most common activities undertaken to support their CPD needs. Generally, respondents preferred to involve in activities that they think they will enjoy, and activities that they can do in their own time and at their own pace. The three main barriers when pharmacists selecting training activities were having commitment outside work, limited time and high workload. Activities that enhance performance in pharmacists' current role were the most important motivating factor for respondents to engage in CPD. Respondents thought that CPD needs can be identified by participating in training courses and through work experience. Group-based learning styles, distance e-learning and multi-professional involvement learning activities were suggested by the respondents in the study.

Respondents used a variety of learning activities to support their CPD needs. Most pharmacists in this sub-population were working full time and were aged in their 30's or 40's, therefore family and work commitments may explain why majority of respondents preferred independent learning activities which allowed them to learn in their own time and at their own pace. Furthermore, inconvenient times and locations were also concerns for pharmacists when selecting training activities. Greater understanding of pharmacists' learning styles and preferences may help in the design of training activities to support CPD needs.

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Effect of moisture on pressurised metered dose inhaler performance by Andersen Cascade Impaction

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This study investigated the effect of moisture and temperature on the stability of pressurised metered dose inhalers (pMDIs). It is known that moisture permeation of pMDIs will increase as temperature and humidity rise, but there is little published data as to how this will ultimately affect inhaler performance. pMDIs are the most widely used respiratory device¹ and these inhalers once dispensed are often stored by a patient in their home bathroom. As a result stability, when introduced to higher temperature and moisture conditions, is essential to ensure reproducibility and clinical efficacy. Previous studies have shown that moisture content can affect formulation stability of aerosols². It is thought that the moisture could lead to aggregation or crystal growth, otherwise known as Ostwald Ripening³. As a result the mean particle size would increase and this would lead to a change in the particle deposition pattern. In addition it is known that the new HFA propellants have a higher affinity for moisture than CFC propellants⁴ so it is thought that HFA pMDIs may be more prone to water ingress and eventual water content. The aim of the study was to determine the effect of high temperatures and moisture levels on the performance of pMDIs. In order to quantify this effect, calculations of fine particle fraction (FPF), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were carried out.

In this study Ventolin HFA and Airomir inhalers were investigated. In addition new formulations were created using pressure filling. The first contained 14% ethanol and 0.14% oleic acid, and the second containing 10% ethanol and 0.01% oleic acid. For each formulation nine canisters were produced, six of these being spiked with water. This allowed the comparison of absolute, 98% and 96% ethanol. All of the pMDIs were stored under accelerated storage conditions of 40°C and 75% relative humidity. The Ventolin and Airomir formulations were tested every seven days and the novel formulations were tested every ten days due to time constraints. The Andersen Cascade Impactor was used to determine particle deposition and to produce an aerodynamic particle size distribution. High performance liquid chromatography was chosen as the method of analysis, and the peaks produced integrated to produce values for FPF, MMAD and GSD. All results produced were statistically analysed using the SPSS software package.

Results showed that the FPF for Ventolin significantly reduced from 50.41% to 24.63% by week 4 of storage. For the Airomir formulations the change in FPF was smaller but still significant by week 3 of storage. FPF for the novel formulations did not change significantly during storage, regardless of the water content present. The only formulation that showed a significant change in MMAD was Ventolin HFA. It is thought this was due to particle size aggregation or crystal growth. This change in size could lead to a change in clinical efficacy as the drug particles will be delivered to a different part of the respiratory tract.

In conclusion, it has been shown that moisture can play an important role in aerosol performance. The resulting increase in particle size leads to a change in particle deposition. This means less is reaching the respirable region and desired site of action therefore the formulation may be less efficacious. The results obtained show the importance of controlling both moisture and temperature in order to preserve stability and to ensure shelf life and clinical effectiveness is maintained.

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A study into the effects of 5-hydroxytryptamine on ischaemia-reperfusion injury in the isolated left atrium

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Myocardial ischaemia can lead to infarct tissue if left untreated. The only successful treatment is reperfusion, which unfortunately also damages the myocardial tissue. When tissue is damaged by reperfusion it is termed Ischaemia-Reperfusion (I/R) injury. In order to protect against I/R injury an intervention must be administered directly at reperfusion. 5-HT has commonly been thought to exacerbate I/R injury¹ however one study found that when applied exogenously it could be protective². The aim of this study is to find out if 5-HT has a protective effect on I/R injury in the isolated left atrium.

The left atrium was dissected and mounted in an organ bath containing Krebs solution and gassed with 95%O₂/5%CO₂. It was electrically stimulated and allowed to contract until a baseline developed tension of >0.25g was reached. Ischaemia was then simulated by switching the bathing medium to Glucose-free Krebs and gassing with 95%N₂/5%CO₂ for 45 minutes.. A vehicle control or 5HT at concentrations of 0.05µM, 0.1µM or 1µM was added immediately after reoxygenation and the bath was washed out at fifteen minute intervals. Developed tension was measured at specific time points throughout. Statistical comparison was made with repeated measures ANOVA followed by Dunnet's post-hoc test, with a p-value<0.05 considered statistically significant.

5-HT at a concentration of 1µM was found to produce a statistically significant improvement on recovery. Although not statistically significant at lower concentrations a trend to concentration dependency was seen. No drop in contractile function was seen when 5-HT was washed out at fifteen minutes. A benchmarking experiment where 5-HT was applied to non-ischemic tissue found that 5-HT at a concentration of 0.05µM had no effect on contractile function.

Findings support that 5-HT promoted contractile recovery in this model. This could be cardioprotective against I/R injury. They also suggest that this protection may occur in a concentration dependent manner. The lack of drop in function when 5-HT is washed out and no change in contractile function in non-ischaemic experiment show that 5-HT activity in this model is in a manner independent of its inotropic effects.

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Effects of the anti-cancer nucleoside analogues, cladribine and clofarabine on the growth of breast cancer cell lines

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Breast cancer is the most common form of cancer in women. The presence of the oestrogen receptor (ER), in particular ER a is one way in which the cancer is categorised¹. The majority of current therapies, such as tamoxifen, target the oestrogen receptor and therefore have little benefit in the treatment of the oestrogen receptor-negative (ER-ve) form. ER-ve cancer has been associated with a poorer prognosis and is a more aggressive form of the disease². Cladribine and clofarabine are nucleoside analogues of the purine, deoxyadenosine, currently used in the treatment of forms of leukaemia. Clofarabine is a second generation nucleoside analogue developed to include the activity of cladribine, however enhancing other characteristics such as its susceptibility to inactivation and acid stability.³ The drugs require transport into the cell via nucleoside transporters and, once inside the cell, undergo sequential phosphorylation to their tri-phosphate, active form. The initial phosphorylation is carried out by the cytosolic enzyme deoxycytidine kinase (dCK)⁴. This is the rate-limiting step in the activation of the drugs. The aim of this project was to investigate whether cladribine and clofarabine affected the viability of ER-ve cells. The project also aimed to determine the mechanism of action of the nucleoside analogues, their method of entry into the cell and the mechanism of cell death caused by the drugs. The activity of the respective protides of the nucleoside analogues were investigated to determine which was superior in the ER-ve cell line.

Experiments were performed in MDA-MB-231 cells (ER-ve breast cancer cells), and HL-60 cells, (a leukaemia cell line). Comparisons were made to results obtained previously in MCF-7 cells, an ER-positive (ER+ve breast cancer) cell line. MTS assays were performed on the cells in order to determine the percentage reduction in viability caused by various drugs. AnnexinV/Propidium Iodide (PI) staining was used to determine the mechanism of cell death caused by cladribine and clofarabine.

Cladribine and clofarabine were most potent in the HL-60 cells and had little activity in the MCF-7 cells. Against the MDA-MB-231 cells, cladribine had a similar potency to that observed in the HL-60 cells. Both cladribine and clofarabine reached a plateau in their response in the MDA-MB-231 cells before causing a steep reduction in viability at 300 μ M. The drugs required phosphorylation in order to gain activity as pre-treatment with the natural substrate of dCK, deoxycytidine, inhibited the anti-cancer activity of both drugs. Pre-treatment with dilazep, an equilibrative nucleoside transporter inhibitor, did not inhibit the anti-cancer activity of the nucleoside analogues indicating that the drugs entered the cells via an alternative mechanism. At 1 μ M, the nucleoside analogues caused a significant reduction in viability, unlike their respective protides. A significant reduction in viability by the protides was observed, however, at higher doses. Annexin V/PI imaging indicated that death due to the drugs was via apoptosis.

The differences in potencies observed between the cell lines could possibly be due to differing levels of dCK and 5' nucleotidase (5'NT), the enzyme responsible for dephosphorylation of the nucleoside analogues, present within the cells. The HL-60 cell could potentially have a higher level of dCK and as a result activate more of the drug, and a lower level of 5'NT therefore less drug is dephosphorylated and remains active for longer. The plateau in response observed in the MDA-MB-231 cell line to the nucleoside analogues could be due to saturation of dCK, the enzyme responsible for the initial phosphorylation of the drugs. The increased reduction in viability observed at higher doses, however, could be as a result of the drugs becoming phosphorylated by other kinases present within the cell. Alternative mechanisms of entry into the cell could include facilitated entry via other transporters such as the concentrative nucleoside transporters or entry via passive diffusion. The lower potency of the protides could be due to low levels of enzymes responsible for cleavage of their protecting groups. This therefore means if no cleavage of the protecting groups occurs, the drug remains inactive. The increased potency of the protides at higher doses could be as a result of more efficient passive diffusion into the cell as a steeper concentration gradient has been achieved. The Annexin V/PI staining technique indicated that cell death was due to apoptosis as both green and red staining was observed in the treated cells suggesting that the cells were in the late stages of apoptosis. In conclusion, this project has identified a potential use for the nucleoside analogues, in particular cladribine, in the treatment of ER-ve breast cancer.

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The identification of adverse drug reactions (ADRs) in a district hospital setting

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Adverse Drug Reactions (ADRs) cause a significant burden on the National Healthcare Service (NHS) and the available literature relating to their incidence within hospitals or causing hospital admission is considerable. However, many UK based studies were performed around 10 to 30 years ago on relatively small scales and limited to individual hospital units.¹⁻³ A recent study conducted in the Merseyside area has provided an estimation of the number of hospital admissions resulting from ADRs.⁴ Differences between study design methods and hospital specialities have resulted in a wide range of the reported ADR incidence in the literature. The pharmacy department at Glan Clwyd hospital identified the problems associated with ADRs as a topical area of research. The primary aim of the study was to prospectively describe ADRs identified by hospital pharmacists which caused admission, prolonged inpatient stay, involved persistent or significant disability or incapacity and of medical significance. The second aim was to identify the number of ADRs reported to the MHRA on Yellow Cards during the study period.

The data collection period was between 16th February and 20th March 2009. The methodology involved the use of a data capture form based on aspects of the Yellow Card system, collecting anonymous information relating to the patient, the suspected drug and reaction, medical conditions, laboratory results and Yellow Card reporting. The inclusion criteria for ADRs were those resulting in hospitalisation or occurring during inpatient stay identified between Monday and Friday. Cases involving drug abuse, alcoholism, overdose, errors in administration, and non-compliance were excluded, as were ADRs occurring on the psychiatric unit, cancer unit, Accident and Emergency (A&E) department and five wards with no pharmacist cover.

During ward rounds, the pharmacists identified 97 patients with ADRs, where 48.5% were reported from the Acute Medicines Unit (AMU). Of the patients identified with ADRs, 71.0% were classed as potential and 28.0% were as definite ADRs. The other 1% were categorised as unlikely ADRs. Of the patients reported, 60.8% were female, 79.4% were aged 65 years or over and 58.7% had three or more relevant medical conditions. The results revealed the most commonly implicated drug classes to be diuretics and antibiotics, followed by a group comprising of anticoagulants, antiplatelets and fibrinolytics. The most frequent drug-induced reactions were hyponatraemia and bleeding, where bendroflumethiazide and aspirin were the most common drugs responsible, respectively. Just over half of the ADRs reported caused hospital admission and further categorisation by the pharmacists revealed that 8.8% had prolonged hospitalisation, 3.1% involved persistent or significant disability or incapacity, and 34.5% were of medical significance. In total, 94 of the 97 cases were categorised by the pharmacists in accordance with the Yellow Card system and so may be considered as serious reactions eligible for reporting to the MHRA. However, only 14 Yellow Cards were completed and reported.

The study successfully identified a significant number of ADRs leading to hospitalisation, with similarities to other UK based studies.⁴⁻⁶ This suggests measures need to be implemented to reduce the ADR incidence causing hospital admission. The results highlight that the hospital may need to consider the problems associated with Yellow Card reporting and develop strategies focused on its improvement. The findings on ADRs and Yellow Card reporting have been presented to the pharmacists at Glan Clwyd hospital. Limitations to the study which should be considered when interpreting the data include the short data collection period which was only at one point in the year and in one hospital. The research has provided the pharmacy department with an opportunity to consider the results obtained and to extend the project through a retrospective review of patient notes to form causality, seriousness and preventability assessments. Further work could include conducting prospective studies in Wales or UK based hospitals over a 12 month period, to provide comparable data relating to the burden of ADRs on the NHS. This could be used to develop ADR preventative strategies for implementation into both primary and secondary healthcare sectors.

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A qualitative evaluation of student use of the self-needs analysis tool in the Cardiff University MSc in Clinical Pharmacy

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The MSc in Clinical Pharmacy is a postgraduate course for hospital pharmacists. It is run in five centres across England and Wales, namely Bradford/Airedale, Oxford, South East Wales, South West Wales and North Wales¹. In 2005 the course was restructured after consultation with stakeholders². The need for graduates of the course to be more independent emerged from this consultation. In an attempt to create this independent attitude self-needs analysis tools were added to a number of the modules. These tools comprise of a series of learning outcomes, the students must decide if the learning outcomes are met, unmet or in-between at the beginning of each module³. Both the students and tutors are expected to use the tools to guide learning throughout the modules. However, it is not known how the students are currently using the self-needs analysis tools. Therefore the aim of this study was to evaluate students' use of the self-needs analysis tool used in the MSc in Clinical Pharmacy.

Preliminary interviews with two Associate Course Directors were carried out to establish what members of the Course Management Committee knew or suspected about use of the tool. A focus group schedule was developed from these discussions. Five focus groups were conducted, one in each centre that runs the course. Invitation emails were sent to 47 second year students, 20 students responded positively to this email (one subsequently dropped out) and 14 students expressed interest in participating on the day. In total 33 students participated in focus groups with 5-8 participants in each group. The discussion was audio recorded and an assistant moderator was present to make field notes. The recordings were transcribed verbatim and analysed thematically.

It was found that students used the self-needs analysis tools at the beginning of the modules but did not use it again. Barriers such as time and lack of opportunity prevented the tools from having a significant effect on their learning. The students didn't understand the task, in particular what level of competence was expected of them. They liked the way the tools could act as frameworks but disliked the repetition and generality of the tools. They praised any that felt specific. They suggested two major improvements to the tools, having one for each year or making them more specific to their working environment. According to the students the tutors weren't using the tools; overall the students felt the tutors should be more involved in the task.

This study confirmed what was previously suspected regarding student use of the tool. It also highlighted a number of ways students' use of the tool could be improved. Recommendations include reiterating to tutors the importance of their role in the task and providing the students with better explanation of the task. More long term the Course Management could review the structure of the tool and the way it is made use of in the course. The main limitation of this study is that it only considered the opinions of students; other users of the tool such as tutors were not consulted. Therefore any conclusions drawn here are premature and would benefit from further study in the form of exploratory research into the views of tutors and Associate Course Directors.

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Design and presentation of a computer assisted learning package on hypertension – clinical features, investigations and pharmacology

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The aim of the project was to design a computer-assisted learning (CAL) package, using Microsoft PowerPoint®, on hypertension to include: clinical features, investigations and pharmacology. This was then presented to MPharm II students, who have recently been introduced to the condition, to obtain their views. Hypertension is a very common condition and is a major risk factor for the development of cardiovascular disease¹ as well as leading to end-organ damage. However, the early recognition and the implementation of effective non-pharmacological and pharmacological treatment options allow modification of such consequences. Pharmacists play a role in the prevention, early diagnosis and management of hypertension², it is therefore essential that pharmacy students have an excellent foundation to the condition to ensure this.

CAL is an interactive computer-based method of learning, proved to be a popular and effective tool in education providing a number of advantages³. The CAL package aimed to provide a detailed overview of all pharmacy-related aspects of hypertension appropriate to the target audience. In order to provide this information the use of colour, animation, visual aids such as images, tables and diagrams and case-studies were applied to maintain interest of the student aiming to enhance their learning. To obtain the views of MPharm II students on the package as a whole, its presentation and content as well as the use of CAL, a 5-point Likert Scale questionnaire allowing a response of strongly agree, agree, no opinion, disagree and strongly disagree, was developed. To overcome limitations of the Likert Scale, a faculty for free text entry was provided. The Likert Scale responses were inserted into a statistical analysis package, SPSS, for statistical analysis to be done and the comments provided were discussed alongside.

Twenty five questionnaires were returned by MPharm II students, providing a response rate of 21.55%. In general there was a very positive response to the hypertension CAL package, 96% finding the package beneficial and useful with comments such as '*...one of the best CAL packages I have viewed...*' and '*...would be very useful to have more CAL packages like this...*' being provided. With regards to presentation of the package, 92% believed it was well presented. However three main issues were addressed: the need for more visual aids through diagrams and images, the slight over-use of animation was referred by a few students as well as there being some controversy in the use of colour. These will be taken into consideration for further improvement of the package and the development and improvement of other such packages and presentations. During development, length was an issue. However as it is a learning package and not for supplementation, it needs to be comprehensive therefore no information could be omitted or reduced. It was therefore pleasing to find that MPharm II students realised this, 88% believed the package was of appropriate length for the content covered. With regards to content, 96% believed that the content covered was appropriate for the purpose of the learning package with no negative responses obtained. The package was aimed towards second year pharmacy students therefore two things needed be taken into consideration. Firstly, the need to ensure the appropriateness of the terms used and the way the information was provided: comments such as '*...in our calibre...*' and '*...excellent terminology...*' were provided. Secondly, that the information provided relates to the pharmacy profession, no student believed that too much inappropriate or irrelevant information was provided for pharmacy students.

With regards to CAL as a learning method, 88% believe it is effective however no student thought it should replace the more traditional learning methods and that a combination of both would be most beneficial.

A thorough CAL package on all pharmacy-related aspects of hypertension, which is easy to read, understand and follow, has been created and has been of benefit to a number of MPharm II students.

It was concluded that teaching staff of the MPharm degree at the Welsh School of Pharmacy should 'consider' the need for the development of new and improved learning packages which are easier to follow and provide more detail accompanied by higher awareness of available packages. However, due to limited response of MPharm II, the project should be repeated to improve response, as well as being accessed by all four years of the pharmacy degree course to ensure that the views observed are representative, prior to drawing further conclusions and mainstreaming such learning packages within the undergraduate course.

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Regulation of L1CAM expression in MCF-7 cells

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Breast cancer is the most common cancer in England, and is the second most common cause of cancer death in women, after lung cancer¹. Since oestrogen receptors play a central role in the growth and development of the majority of breast cancers, ER-positive patients are commonly treated with antioestrogens such as tamoxifen in addition to surgical intervention. Unfortunately although very effective, a high proportion of initially-responsive patients eventually acquire resistance to tamoxifen treatment. Faslodex is a potent competitive inhibitor of oestrogen that works by degrading the ER and is used in tamoxifen resistant patients². However, like tamoxifen, resistance to faslodex may also develop. Interestingly, in vitro models of faslodex resistance (FasR cells) have been found to be ER-negative and to overexpress L1CAM (L1 adhesion molecule), a protein whose expression promotes the scattering of the epithelial cells³ and is associated with cellular invasion and migration. It is therefore crucial to understand how L1CAM is up-regulated in breast cancer cells and whether this involves the ER.

The western blotting technique was used to determine L1CAM expression in MCF7 Cells following treatment with estrogen (10^{-12} M), tamoxifen (10^{-7} M), faslodex (10^{-7} M) or AZD0530 (1 μ M) or under oestrogen-deprived conditions (to mimic aromatase inhibitor treatment) for three days and ten days. After lysing in lysis buffer containing protease inhibitors, the total soluble protein was resolved in a SDS-PAGE. The proteins were then transferred from the gel into a nitrocellulose membrane, which was then blocked and probed for three different primary antibodies namely total ER (66kDa), p-ER118 (66kDa) and L1CAM (200-220kDa). A rabbit secondary antibody was used to bind to Total ER and p-ER118, whereas a mouse secondary antibody was used to bind to L1CAM. The protein levels were detected using a chemiluminescence technique and X-ray films. Immunofluorescence was used to confirm the expression levels of L1CAM that were observed by the western blotting. Cells were incubated with L1CAM antibody and Ankyrin G. The cells were then incubated with a mixture of two fluorescent secondary antibodies; the green Goat anti-mouse 488 (GAM488) for L1CAM and the red Goat anti-mouse rabbit (GAR594) for Ankyrin G. The cells were mounted using Vectorshield aqueous mountant plus the DNA stain 4', 6- diamidino-2 phenylindole (DAPI) to facilitate the detection of all present cells in the slide even in the absence of the target antigen. The slides were visualised using Leica inverted fluorescent microscope coupled with CCD camera and captured using Openlab software.

Compared to untreated MCF7 cells, Faslodex and estrogen treatments greatly reduced expression of the estrogen receptor whereas estrogen deprivation, tamoxifen and AZD0530 treatments slightly induced the expression of the ER. With regards to the ER activity (phosphorylation at Serine 118), tamoxifen induced this to the greatest extent, with a modest increase seen with faslodex, estrogen and AD0530. Estrogen deprivation showed the lowest activity levels. The results also revealed that faslodex induces the highest expression of L1CAM and at both short (3 days) and long (10 days) treatment times. Although the other treatments seem to induce L1CAM expression initially this was not observed with the long term treatment. On the contrary, estrogen stimulation and tamoxifen treatment reduced L1CAM levels by 80% and 70% respectively.

Although antihormones are the mainstay of breast cancer treatment and have many benefits, it is becoming apparent that they have the ability to induce the expression of elements which may promote adverse cell behavior. Although a direct correlation between ER expression and/or activity and L1CAM levels was not observed, L1CAM expression was greatly enhanced by faslodex compared to all other treatments. Given that both oestrogen and faslodex downregulated the ER, these data suggest that ER-mediated gene expression suppresses L1CAM expression in MCF7 breast cancer cells.

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Medicines Use Reviews and the Welsh language – Patients' views

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Effective communication is essential for the successful provision of healthcare¹. In Wales over a fifth of the population speak Welsh² and although most are also able to speak English, research^{3,4} has determined that Welsh-speakers feel happier and more confident communicating with healthcare professionals through the medium of Welsh. Indeed, not providing a language choice for such patients may be detrimental to the development of the healthcare professional-patient relationship and ultimately to the quality of care provided³. The Medicines Use Review (MUR) service was introduced as part of the 2005 Pharmacy contract to encourage pharmacist and patient to work together to improve a patient's understanding and use of their medicines⁵. Currently, the pharmacist may choose to conduct the MUR consultation through the medium of Welsh however, despite recent revision of the form it is not yet available in Welsh meaning that the pharmacist must on-the-spot translate to fill in the required documentation, and no language choice is available for a Welsh speaking patient. This study thus aimed to determine Welsh speaking patients' language preferences for the MUR consultation and form, and to explore the reasoning behind these preferences.

The research was exploratory hence the qualitative approach of semi-structured interviews was utilised for data collection. Purposive recruitment, of Welsh speaking patients who had recently had a MUR, was undertaken from two sites in Carmarthenshire, an area with a high proportion of Welsh speakers. Site A was an independent community pharmacy and site B part of a large chain multiple. Sixteen responded to the recruitment letter but unfortunately, difficulty arranging interview times and dates meant that not all participated in an interview. Three pilot interviews with volunteers matching participant criteria were initially undertaken. Nine interviews in total were subsequently conducted with recruited participants, eight audio-recorded and transcribed verbatim (one refused consent), and all thematically analysed.

Despite feeling more confident when speaking English, due to a perception of their Welsh 'not being good enough', all those interviewed stated themselves to be most comfortable when speaking Welsh. Similarly, it was discovered that consultations through the medium of Welsh occurred naturally between Welsh speaking patients and their Welsh speaking pharmacists, and consequently most felt that their language of choice for the MUR consultation would also be Welsh. However, if faced with being unable to speak Welsh most would willingly have the MUR in English and would not request to speak to a Welsh speaker as they were also able to speak English and for fear of 'making a fuss'. In terms of the MUR form, although not averse to it being bilingual, all felt that they would want it completed through the medium of English and references were made towards not understanding some words in the more formal written Welsh. Concern was also expressed that non-Welsh speaking doctors would be unable to understand the form if completed in Welsh.

Although limited to the views of a small number of patients in Carmarthenshire, overall it can be concluded that these Welsh speaking patients felt that the MUR consultation being in Welsh was of greater importance to them than the form. While further research is still required to determine the extent of the need for Welsh language provision of the MUR service across Wales, it appears that Welsh-speakers believe that being able to speak Welsh during the consultation is important to them. Consequently pharmacists and pharmacy staff should be aware of this and, wherever possible, a language choice provided for Welsh-speaking patients.

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An investigation into the potential interaction between the small GTPase Rab7b and the autophagy protein ATG

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Unpublished data produced by the Jones laboratory has discovered evidence that shows the small GTPase Rab7b is upregulated in multidrug resistant HL60 leukaemia cells. These MDR cells lines have been extensively studied revealing major differences in the endocytic pathways of drug resistant and drug sensitive cells.¹ It is undetermined whether Rab7b upregulation has any role to play in multidrug resistance and to date no binding partners for the protein had been discovered. On these grounds a cDNA plasmid encoding for Rab7b was sent to the DKFZ German Cancer Research Centre for analysis of binding partners by yeast two hybrid analysis. A yeast two hybrid screen with a bone marrow library revealed an autophagy protein ATG to be a putative Rab7b interacting protein. Rab7b is involved in the process of endocytosis and has been shown to be potentially involved in the regulation of protein transport by facilitating a docking and fusion process.² ATG is a protein used as a processor and de-conjugating enzyme for autophagosome forming modifiers in the process of autophagy. Autophagy is a process by which endogenous proteins and damaged organelles are destroyed intracellularly.³ Autophagy is a degradation pathway that allows the cell to eliminate unwanted organelles and then recycle the components for re-use. The information obtained from the German Cancer Research Centre suggests a link between Rab7b mediated endocytosis and the process of autophagy and therefore formed the template for this study which aims to test the predicted interaction between Rab7b and ATG.

A549, HeLa and KG1a cells were examined by western blotting in order to detect ATG and Rab7b. Following these experiments HeLa cells were transfected with a Rab7b-GFP cDNA plasmid using Fugene6 transfection reagent in order to increase Rab7b levels within the samples. After transfection samples were viewed under a confocal microscope to confirm the transfection was successful. Rab7b-GFP transfected HeLa cells were subjected to western blotting to detect ATG, Rab7b and GFP. Once these proteins had been detected by western blots transfected HeLa cells underwent immunoprecipitation using anti-GFP antibody and a control of anti-Clathrin Heavy Chain antibody and Protein-G on cross-linked 4% beaded agarose. The immunoprecipitate was then examined by western blotting in order to ascertain whether the experiment had been successful in precipitating Rab7b-GFP out of solution and then to investigate whether a binding interaction between Rab7b and ATG had resulted in ATG being isolated in the immunoprecipitate.

Western blots detected the relevant bands expected for ATG, Rab7b-GFP and CHC using the appropriate antibodies in A549, HeLa, KG1a and transfected HeLa cells. Rab7b was undetectable in A549, HeLa and KG1a cell samples via western blotting using a series of anti-Rab7b antibodies. Anti-Rab7b antibodies were able to detect Rab7b-GFP in transfected HeLa samples. The transfected HeLa cell samples that underwent immunoprecipitation failed to produce any bands following western blotting. Samples of the transfected HeLa cells taken before the immunoprecipitation took place produced bands when probed with anti-GFP and anti-CHC antibodies, indicating that the immunoprecipitation experiment was unsuccessful and failed to precipitate Rab7b-GFP and CHC.

It was proven by Western Blotting that ATG, Rab7b-GFP and the control protein clathrin heavy-chain were detectable utilising the appropriate methods. Thus we can conclude that the next step into investigating the potential interaction between Rab7b and ATG would be to conduct an immunoprecipitation whereby Rab7b-GFP is immunoprecipitated by Protein-G agarose beads, hopefully immunoprecipitating ATG along with it. While the immunoprecipitations carried out in this study proved to be inconclusive, the unpublished data regarding the yeast two hybrid analysis indicate there is a potential interaction between Rab7b and ATG. An interaction between these two proteins is highly feasible due to their respective roles in the cell. I believe further analysis would result in conclusive evidence of Rab7b playing a role in autophagy

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Differential effect of caveolin-1 expression on pathological features in p53 mutated U373-MG and p53 wild type U87-MG glioblastoma cell lines

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Brain tumours represent 1.6% of all forms of cancers diagnosed in England and Wales, with the Glioblastoma Multiforme (GM) being the most common. The location and aggressiveness of brain tumours coupled with limited treatment options results in a very poor prognosis. 29% of patients live 1 year post diagnosis and a mere 13% live 5 years post diagnosis¹. The role of Caveolin-1 (cav-1) in cancer progression is both complex & contentious. The expression of cav-1 has been assessed and modulated in a number of different tumours but to date no clear trend has emerged². In some cancers it appears to promote progression whilst in others it inhibits progression. It is believed that cav-1 is interlinked with the highly complex signalling cascades AKT/mTOR and the ERK pathway³. The p53 is a specialised protein which acts as a tumour suppressor, and when functioning correctly provides protection against developing cancers. Unfortunately mutations in the p53 gene are seen in 30% of glioblastoma cancers, these cancer phenotypes tend to be more aggressive and more resistant to chemo-toxic treatments⁴. This study is aimed to establish the true effect of the signal transduction pathways on cav-1 expression in brain tumour cell lines and also to explore the difference in caveolin-1 contribution on the patho-biology of p53 mutated cell line and p53 wild-type cell lines.

During this study the cell lines examined were the p53 mutated (mt) U373-MG and the p53 wild-type (wt) U87-MG. The cells were seeded at 30000cm⁵ in either 6 well (western blotting) or 24 well (cell counts) plate format and grown for 48 hours. The cells were then either treated with 0,1,10,100 nM of Rapamycin or 10, 25, 50µM of ERK inhibitor PD9810. The cells were then either used for viability assays using a coulter counter machine or harvested for western blotting. Some cells were also grown in a 6 well plate format and 20,000 cells were used in order to carry out cell motility assays. Modulation of caveolin-1 expression was achieved using siRNA transfection. Two different sequences were used, K-Cav delivered in a lipofectamine carrier was used to down-regulate cav-1 expression and Luciferase was used as a lipid control. The siRNA transfected cells were used for viability assays, western blotting and invasion assays.

The study found that the two p53 status cell lines responded very dissimilar to each other. The p53 mt U373-MG seemed to be resistant to the inhibition of growth by Rapamycin with only a maximum of 25% growth inhibition. In contrast the p53 wt U87-MG cell lines growth was dramatically reduced by the Rapamycin. The growth inhibition of both cell lines were similarly inhibited by the PD9810 however no dose dependant response was visible. Western blots also provided interesting results highlighting the positive relationship between caveolin-1 and the activity of the ERK pathway in p53 mt U373-MG cell lines. The p53mt U373-MG cells were treated with Rapamycin the activity of the mTOR pathway was obviously decrease however there was an increase in the activity of the ERK pathway which was parallel to the increase in the expression of cav-1. This therefore suggests that there is a possibility that the ERK pathway drives the expression of cav-1. This relationship was not seen in the p53 wt U87-MG cell line, underlining the fact that the p53 status contributes to the patho-biology of the tumour itself. siRNA transfection allowed the examination of varied cav-1 expression. Down regulation of cav-1 brought about a small increase in the level of inhibition of both cell lines growth, suggesting that cav-1 is implicated in the growth signal pathways. The invasion assay results confirmed the difference in patho-biology of cells due to the p53 status, the p53 mt U373-MG had a profound ability to invade which was converse to the very poor ability of the p53 wt U87-MG cell line. However, once again down-regulation of cav-1 did decrease the ability of the p53 mt U373-MG GM to invade once again confirming the implication of cav-1 in the phenol-biology of GM cell lines.

The results show that there is a profound difference in the way that p53mt U373-MG cells and p53 wt U87-MG cells respond to mTOR inhibitors. The p53 status could therefore be the reason for such a large number of glioblastoma being resistant to treatments. The positive relationship between cav-1 and the ERK pathway leads us to deduce that the p53 mt cell lines may have a compensatory mechanism which brings about the acquired resistance towards drug treatments. By further exploring the contribution of cav-1 in different p53 status GM and concreting our understanding of the signal transduction pathways, new drug treatment targets may give the prospect of more effective non-resistant anti-cancer drug treatments which will give patients better prognosis.

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Use of the Medication Related Consultation Framework (MRCF) as a self assessment tool for Pharmacists

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Advances in pharmacy practice have meant that pharmacists need to develop good communication, negotiation and consultation skills. During Medicine Use Review (MUR) consultations pharmacists are well placed to address patient non-adherence to medication. In conducting a successful MUR the pharmacist should adopt a patient centred consultation style meeting the needs for the individual patient, considering factors that may affect the way the consultation is conducted². The Medication Related Consultation Framework (MRCF) was developed by Abdel-Tawab in 2005, as a tool for assessing pharmacist-patient consultations since previous consultation models have focused on doctor-patient consultations. The MRCF combines two components for an effective consultation. Namely the structure and content of the consultation, and how the consultation should be conducted. The Welsh School of Pharmacy's Postgraduate Diploma in Community Pharmacy uses the MRCF as part of the assessment programme where students' self-assess their own consultations following MUR consultations. The aim of this study was to evaluate the use of the MRCF as a self assessment tool. Specific objectives were to investigate how students' rate their own consultation skills, assess how ratings change over time and explore practitioner's views of the MRCF.

Quantitative data were obtained from completed MRCF forms of students enrolled on the Community Pharmacy Diploma since 2005. A database was created on SPSSv16.0[®] using the data gathered from the MRCF forms. Box plots were created to illustrate the change in students self rated score for the overall impression scale, the five global rating scales, and total scores obtained for each of the five individual sections across a series of time points. Paired *t*-tests were used to look for significant differences between the scores. Qualitative data were obtained from two sources. Comments written by students on the MRCF forms were tabulated as strengths and weaknesses. A small sample of students were interviewed. Interview questions focused on gathering students views of the MRCF as a self assessment tool. The data gathered from interviews will be used to design a structured questionnaire to be used in further research.

There were a total of 153 MRCF forms included in the study, representing 63 students. Students completed between one and six frameworks. An increase in students self-rating for the overall impression of the consultation was observed across the time points, from student's initial rating of 'satisfactory' to a final rating of 'good'. Paired *t*-tests showed that the differences between the first and third, first and fourth, and first and sixth MRCF were statistically different $p < 0.05$, two tailed (using SPSS16.0[®]). There was no overall increase in students self rating of three of the global rating scales A (set scene), B (data collection), and C (actions and solutions). A significant increase was observed at global scale D (closing), from student's initial rating at 2.5, to a final rating of 3.0. An increase in total score was observed at section B, scores increased from 17 to 18. Common comments written on the forms related to difficulty in exploring patient's wish for involvement and what to ask. Feedback from eight interviews indicated that students' found it useful for knowing what to include in the consultation. It prompted them to take a full medication history whereas otherwise they may not have done so.

All students interviewed thought the MRCF was a good reflective tool and helped them to develop effective consultation skills. Overall there was an increase in students self-rating of their ability to consult over the two year programme. A decrease in self-rating score was observed at the second MRCF, suggesting that students re-assessed their ability to consult and set a bench mark from which to improve. No increase in self-rating scores were observed at global scale A, students may have perceived themselves to be already competent at introducing themselves to the patient and opening the consultation. No increase was observed at global scale C, the pharmacist's ability to encourage patient involvement and conduct a patient centred consultation did not change over time. Information gathered from the comment boxes support this as students appear to find it difficult to develop a patient-centred consultation. These findings are useful to inform the future learning needs of the postgraduate students and emphasise the need to involve patients in the learning experience.

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An approach for the direct comparison of performance of molecular imprinted polymers

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Molecular recognition between ligand and substrate is of high importance to enable organisms to function properly. Scientists have developed a method of mimicking these types of recognition systems by creating polymers imprinted with sites, which are specific to a chosen substance, forming *molecular imprinted polymers* (MIPS). The application of MIPS is countless, but the main areas that will benefit include the production of biosensors, synthetic antibodies and synthetic enzymes. Other important uses of MIPS over the years has been in *separation studies*, with the majority of these studies involve using liquid chromatography and solid phase extraction. [1] Generally, this type of experiment is cost effective and fairly simply to implement, [2] however MIPS haven't fulfilled their true potential. One of the problems is the lack of uniformity in the publication of MIP experiment findings. Data is presented in a variety of different ways, which leads to difficulties in forming accurate conclusions on the performance of the MIP. Also, data published within journals is selective and therefore certain aspects of the polymers performance may be omitted. [3] The aim of this dissertation is to gather relevant experimental literature and normalise the data in order to arrive at more accurate and reliable conclusions regarding MIP performance. This normalisation of data will enable the evaluation and ranking of the different polymers as well as identifying the effectiveness of the various polymerisation techniques used.

The method chosen in this report consisted of four main steps. The first step involved data research. This was implemented by using a variety of methods including using search engines and a MIP database to find relevant data. This data was then converted into standard units and plotted on a graph to form binding isotherms for each polymer. When a sufficient amount of binding isotherms were created, they could be accumulated on the same graph using *Graphpad Prism*. The last step was to analyse the graphs produced and identify any patterns in MIP performance.

From the graphs key observations could be made. The first observation was that polymers made by *Suspension polymerisation* performed better than polymers of the same template made by *Bulk polymerisation*. Secondly, the use of *Trimethylolpropane trimethylacrylate* (TRIM) as a cross-linker enhances polymer function. In terms of the form of polymer, it was found that polymers of the same template performed better in the form of micro spheres in comparison with *monoliths*. Also, *bead* polymers produced very good isotherm values. The functional monomer MAA worked best in combination with TRIM. Furthermore, the use of MAAP led to very good polymers.

Suspension polymerisation leads to more uniform and spherical polymer particles so it was expected that they would perform better than *monoliths*. This could be due to the fact that the spherical particles would disperse more evenly throughout the assay solvent. Also, due to their uniform shape, the ligand could get easy access to the binding site compared to monoliths. The enhanced polymer binding whilst using TRIM can be explained by its' chemical structure. TRIM is a tri-functional molecule and has more binding sites available compared to the other cross-linkers, which only had two. Therefore, the TRIM cross-linker would form a stronger polymer complex. The level of binding was higher when the polymer was made in the form of micro spheres for a couple of reasons. Firstly, the spherical shape enabled the polymer to disperse evenly throughout the solvent. Secondly, the spherical shape reduces the friction between the surface of the assay and adjacent polymers.

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Synthesis of novel CYP26 inhibitors for neuroblastoma therapeutics

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Neuroblastoma is a malignancy of the cells which usually give rise to the sympathetic nervous system^[1]. It is the most common extracranial pediatric neoplasm and the third most common pediatric malignancy after leukemia and central nervous system (CNS) tumours. However, fewer than 100 children are diagnosed, annually, with neuroblastoma in the UK.^[2] Prognosis depends mainly on primary site of tumour, age at diagnosis and stage at diagnosis.

Retinoic acid (RA), a naturally occurring derivative of vitamin A, causes differentiation of neuroblastoma cell lines, characterised by the appearance of long neurites and cell growth arrest^[3]. RA exerts its activity by binding to transcription-regulatory factors in the cell nucleus – RAR and RXR^[4]. Tests have shown that the presence of the enzyme, CYP26, which regards RA as its main (and possibly only) substrate, causes rapid metabolism of RA to the inactive 4hydroxy-RA. Thus RA has a shortened half life and reduced potency when administered systemically. This led to the development of CYP26 specific inhibitors to improve efficacy of retinoid treatment.

This work describes attempts to synthesise potential novel CYP26 inhibitors based on a general template and varying terminal groups and/or side chains. The chosen template was inspired by the promising initial data of naphthalene-2-ylaminophenyl-1,3,4-oxadiazoles.

Future work will include the optimisation of the products actually or partially synthesised, leading to biological testing of the compounds to determine CYP26 inhibitory activity.

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Effect of BK channel modulators on osteoblast mineralisation

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Osteoporosis is characterised by decreased bone mineral density and skeletal microarchitecture deterioration,¹ which could potentially be reversed by increasing osteoblast mineralisation. Previous studies have suggested that potassium channels play a role in cell function and proliferation in myoblasts and lymphocytes.^{2,3} Further studies have suggested that mineralisation in osteoblasts may be increased via blockade of potassium channels: specifically BK potassium channels.⁴ The aims of this study were to determine the presence of BK channels in primary human osteoblasts and determine the effect of selective BK channel blockers and openers on mineralisation in osteoblasts and osteoblast-like cells. The effects of the blockers and openers on cell number and biochemical mineralisation markers were determined, in order to establish whether changes in mineral deposition were due to altered mineralisation or altered cell number.

The presence of BK channels in HOB-c primary human osteoblasts was tested using RT-PCR. The effects of blockers tetraethylammonium (TEA), iberiotoxin, paxilline, tetrandrine and BK channel opener NS1619 on mineralisation in SaOS-2 osteosarcoma, 7F2 osteoprogenitor and HOB-c primary human osteoblast cells, was determined by staining mineral deposits with Alizarin Red S. Interleukin-6 (IL-6) produced during mineralisation by 7F2 cells was measured using ELISA and the effects of drugs on cell number was measured using MTS assays. Haemocytometer counting of Trypan Blue-dyed cells allowed non-viable cells to be distinguished from viable cells, so that percentage viability could be calculated.

RNA for all BK channel subunits was shown to be present in HOB-c cells. Paxilline and tetrandrine caused a decrease in cell number but no change in cell viability at low concentrations, but killed cells at high concentrations. MTS assays showed no significant difference in cell number with up to 10 mM TEA, 100 nM IbTX, 1 µM paxilline and 10 µM NS1619. There were no major differences in mineralisation in drug treated cells compared to controls, but unexpectedly, 10 mM TEA appeared to stimulate adipogenesis in 7F2 cells. ELISA showed no difference in IL-6 production with paxilline and NS1619 treated cells compared to controls.

The identification of BK channel subunit RNA in HOB-c cells confirmed previous findings. TEA did not appear to increase mineralisation in HOB-c cells, which is contrary to findings of previous studies,⁵ although this may be because cells were not fully mineralised at the time of staining. This does however, cast doubts on the reliability of mineralisation results in cells treated with selective BK blockers. Cells lifting off the well plate due to over-confluence affected the 7F2 mineralisation results. However, TEA did appear to produce adipogenesis in 7F2 cells, which was unexpected. IL-6 is a non-specific marker of bone turnover, and more reliable results could be achieved by measuring osteocalcin or bone-specific alkaline phosphatase. In conclusion, the findings of this study were confounded by contamination of cells, lack of repeats due to time constraints, lifting off of cells from well-plates and mistakes made during execution of the methods. However, the differentiation of 7F2s into adipocytes when treated with 10mM TEA, is an extremely interesting finding which could be the subject of further research.

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Assessment of inter- and intra-individual variability in human skin permeation based on tritiated water K_p values and electrical resistance data

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Transdermal drug delivery offers many benefits over the more conventional method of oral drug delivery as it can avoid problems such as first pass metabolism and many side effects. However, variability in skin permeation between individuals, and even within a single person, is a major issue in transdermal and topical drug delivery. Assessment of permeation *in vitro* provides key information when predicting dermal absorption *in vivo*. Tritiated water flux values (K_p) are frequently used *in vitro* to assess skin barrier integrity to eliminate damaged tissues from subsequent studies which assess penetration and dermal delivery of drugs¹. Measuring Electrical Resistance (ER) across such membranes is claimed to be a simpler, quicker, safer and more cost effective method. Limited attempts have been made to quantify variability and most studies have been conducted on a relatively small scale. It is crucial to understand how permeability is affected by variables e.g. patient age, anatomic site, different individuals.

Existing experimental data on human skin permeability, conducted by a single laboratory over an extended period of time was re-evaluated. Abdomen and breast skin samples had been donated by female patients, with informed consent. Numerous epidermal skin sections were prepared from the skin provided and these were tested for water flux and electrical resistance. Skin sections were mounted in Franz cells and tritiated water K_p values determined. Resistance across membranes was obtained by passing a fixed a.c. current across the sections using a Tinsley Databridge. All the data was pooled together in two databases consisting of water permeability $K_p \times 10^{-3}$ (in cm/hr) and ER (in kOhm's). Analysis of these databases was undertaken in order to increase understanding of variability in human skin.

The population of tritiated water K_p values was initially found to be non-normally distributed. However, a logarithmic transformation of the data produced a normal distribution. A Mean K_p of 1.55 cm h^{-1} was calculated. This value was consistent with previous studies in the same field. The entire ER database was found to exhibit a normal distribution and a mean ER of 28.00 kOhm/cm^2 was obtained. This was higher than reported values. However, previous experiments were done on a smaller scale and used lower cut-off values. Regression analysis of variables indicated that there was no correlation between age and skin permeability in both sets of data. A *t*-test confirmed a significant difference between abdominal and breast K_p values ($P = 0.01$) but no significant difference was found between them in the ER data. It was observed from the study that the coefficient of variance (CV%) was smaller in intra-individual data than inter-individual data in both data sets. Therefore it can be deduced from the study that intra-individual variability in skin barrier function was smaller than inter-individual variability.

The outcomes found in this study correlate with those from some previous studies³. However, there are also studies which disagree. Possible reasons for this could be that permeation of molecules of different sizes and physicochemical properties were evaluated, that there were intrinsic differences in the barrier function of the skin samples evaluated, or that the analysis was incorrect.

In conclusion, this field requires further research. Percutaneous studies need to be repeated with a larger number of samples. The ER data only contained a small number of skin sections, exclusively from breast tissue. Extended studies using a broader range of test permeants must be conducted before any firm conclusions be made.

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Adverse drug reactions: A trend analysis of drug-related admissions to Ysbyty Gwynedd

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An adverse drug reaction [ADR] is defined as 'a response to a drug which is noxious and unintended and which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for the modification of a physiological function'.¹ ADRs are known to be a significant cause of morbidity and mortality; research carried out in the United Kingdom [UK] suggests that ADRs are responsible for 6.5% of all hospital admissions and cost the NHS £466 million per annum.² However, no epidemiological study to date has characterised the nature and burden of ADR-related hospitalisations on hospitals in Wales. The aim of the study was to determine the prevalence of ADR-related hospital admissions at Ysbyty Gwynedd and to identify trends in the patients and drugs associated with them.

Ysbyty Gwynedd is a 515-bed district general hospital situated in Bangor which forms part of North West Wales NHS Trust.³ When a patient is admitted to the hospital, details of their admission are recorded in their medical notes. This information is then coded according to the International Classification of Disease, 10th revision [ICD-10] and transferred onto the hospital's computer system for storage. Admissions involving adverse drug reactions are assigned a specific code between Y40.0 and Y59.9 depending on the class(es) of drug(s) thought to be responsible.⁴ By searching for admissions records bearing these codes, details about drugs, symptoms and patients involved in ADRs can be retrieved for analysis. This study retrospectively analysed records of ADR-related admissions between April 1st and October 31st 2008, the most recent date for which complete, computerised records data was available. In order to maintain patient confidentiality, records searches were performed by personnel within the hospital's clinical coding department. Furthermore, all results provided (in printed spreadsheet form) were anonymised. In order to make different individuals distinguishable, each patient had been assigned a random number of between two and five digits. This allowed patients who had experienced multiple ADR-related admissions to be detected.

During the period analysed there were a total of 30,837 admissions to Ysbyty Gwynedd. The records indicated that 246 of these admissions were ADR-related, and it was therefore calculated that ADRs were responsible for 0.8% of all admissions to Ysbyty Gwynedd. These ADR-related admissions had been experienced by just 208 individuals; 33 of these individuals (15.9%) had experienced multiple admissions as a result of ADRs. With regard to the gender of persons admitted due to ADRs, it was found that 57.2% (n=119) were female and 42.8% (n=89) were male; this represented a statistically significant difference between the sexes (p=0.0375). It was also found that persons aged 65 and over were significantly more likely to be hospitalised as a result of ADRs (p=0.0009); the mean age of those admitted was 70 years old (SD = 16.45 years, 95%CI = 68 - 72 years). In all 53 different drug groups were found to have caused ADRs, though NSAIDs (24 ADRs), ACE inhibitors (23 ADRs) and Antineoplastic drugs (23 ADRs) were responsible for the highest number of admissions. Hypotension (29 instances) and renal failure (23 instances) were the most commonly seen adverse effects.

The study succeeded in identifying a number of significant trends within the records of ADR-related admissions to Ysbyty Gwynedd, and its results make a meaningful and useful contribution to the existing knowledge base surrounding ADRs. The observation that ADRs were more common in females and the elderly was in line with the results of previous UK studies, as was the finding that NSAIDs and ACE inhibitors were associated with a high number of admissions.² However, some of the observations made were more unexpected. For instance, it was found that salicylates and drugs with narrow therapeutic indexes contributed to notably few ADRs in spite of their relatively widespread usage and their well-documented ability to cause side effects. Additionally, the prevalence of ADR-related admissions was found to be markedly lower than the previously suggested figure of 6.5%. This may be the result of a genuine trend or may be indicative of some form of ADR under-recording. This is a potential limitation of the retrospective study design used; the results provided by it are only as complete and accurate as the admissions records they are based on. As such, it may be useful to conduct a further prospective study to verify the results offered by this particular study.

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Optimisation of formulations for efficient microneedle coating and drug release in skin

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Many new pharmaceutical products are based on large molecules, for example proteins and DNA,¹ which cannot be delivered via the preferred oral route. These new drugs are often delivered parenterally, but this route currently has many problems including pain and the risk of blood-borne diseases from dirty needles. Therefore a new method of transdermal delivery has been developed – the use of microneedles. These microneedles are too short to reach nerve endings and so are associated with little or no pain.² There are several different types of microneedles, but coated microneedles are perhaps the most commercially attractive option. However, there is little research on the most effective way to coat a microneedle with drug solution or on the optimum release time in human skin. This study's main aim was to find the optimum length of time a coated microneedle array should remain in the skin to obtain the requisite drug release.

Using microneedle arrays, the current dip-coating method was improved upon by creating a novel dipping well. In addition, the optimum number of dips required to fully coat a microneedle was investigated. Different drug-coating solutions were tested using fluorescence as a marker and the amount of Lutrol contained within them varied to find the most effective solution for coating. The arrays were washed with 300µl water and the fluorescence measured. Calibration curves were produced and the fluorescence concentration coated onto the needles was calculated. Pictures were also taken of each coated array under fluorescence microscopy. The microneedle arrays coated in drug solution were placed into skin for 1 minute, 15 minutes and 30 minutes. They were then removed and washed to calculate the amount of drug removed from the microneedles by the skin at these three time-points. The diffusion profile in skin was examined by coating microneedle arrays in a histochemical stain and inserting them into the skin for different time points. Upon removal, the size and intensity of the resulting stain in the skin was examined. To study the effect of needle puncture on the skin, TransEpidermal Water Loss (TEWL) was investigated before and after needle puncture. Microneedle arrays with no coating were also inserted into the skin to investigate whether the coating itself had any effect on needle puncture and TEWL.

Using an improved dipping well achieves more consistent dipping, as well as allowing easier calculation of how much drug has been coated onto a microneedle. The results show the most effective coating solution is one that contains Lutrol, more specifically 0.5%, 2.5% or 5% of Lutrol. Measuring the fluorescence left on the microneedles after skin insertion for 1 minute showed a large amount remaining on the microneedles. 1 minute is therefore not long enough for the coating to dissolve off the needles and longer times are necessary. However, inserting fluorescence coated microneedles into skin was proven to be problematic. Using a histochemical stain to visually study dissolution times was more effective and showed that the longer time needles were left in the skin, the more drug dispersal occurred. TEWL measurements showed that microneedle puncture does increase water loss, but by a small amount. The longer the needles are left in the skin the less that water loss becomes.

In conclusion, coated microneedles are a viable method for transdermal delivery of pharmaceutical products and the current coating process can be improved upon. However the coating solution needs further investigation to achieve optimum release in human skin.

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An investigation into participants' views of the Welsh School of Pharmacy clinical placement scheme

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Traditionally, pharmacy undergraduates have been taught through lectures and workshops, designed to impart knowledge and understanding. However, experiential learning is now increasing as universities realise that clinical skills and problem based learning are important to prepare pharmacy undergraduates for professional life. The Royal Pharmaceutical Society publishes guidance regarding practice placements which states that the student has to gain 'first-hand structured experience of practice, including contact with patients and practitioners of other healthcare professions.'¹ To comply with this, the Welsh School of Pharmacy (WSoP) provides a two and a half day clinical placement, which is spent in a hospital in Wales or the South West with a manual to guide and record activities. This project aims to determine participants' views of the positive and negative aspects of the WSoP's clinical placement scheme and manual.

Semi-structured interviews were conducted to gain initial views and opinions from participants in the placements. Four MPharm III students and two local hospital pharmacists were interviewed. The interviews were audio taped and the opinions categorised² and used to formulate three different semi-quantitative questionnaires. These were hand-delivered to the WSoP facilitators and to students who had completed their placement. Hospital pharmacist questionnaires were posted to all 20 participating hospitals.

The response rates gained were 100% for WSoP facilitators', 92% for students' and 65% for hospital pharmacists' questionnaires. The results obtained from the questionnaires were reflective of those in the interviews. Both students and hospital pharmacists were found to prefer placements in the 2nd and 3rd years, with earlier placements allowing more voluntary experience to be gained before making pre-reg decisions. Students felt that the placements were not long enough at two and a half days to gain a full insight into hospital pharmacy, as most of the time was spent completing the manual and they were unable to participate in many activities because they didn't have the time to complete the necessary training. Hospital pharmacists and WSoP facilitators also believed that longer placements were needed, but they acknowledged that time and financial constraints prevented this. Hospital pharmacists would also like more frequent placements so that the demand is spread out. 40.79% of students thought the manual was too restrictive and were more worried about completing it than enjoying their time, although most hospital pharmacists thought the manual served as a good guide, providing target activities to demonstrate. Positive aspects highlighted by students included participating in ward rounds, meeting patients and observing pharmacist-led initiatives, e.g. prescribing clinics and medicines information. Negative points concerned the length of the placement, time spent idle, travel expenses and aspects of the manual.

This project suggests that the WSoP should aim to increase the length and frequency of clinical placements, incorporating more of the positive experiences highlighted. Participating in more placements is beneficial to students, as they help to foster a professional identity³, as seen in other healthcare students. Placements would allow students to gain more experience in a range of pharmacy sectors, thus assisting their future career choices, as well as improving their communication skills. A review of the manual is needed to ensure it meets the requirements of both students and hospitals. This could include the introduction of more interactive activities which assist students' learning and more guidance for pharmacists on the level of knowledge to expect. Further work could include analysis of subsets of the data, such as those students whose 2.5days was part of a longer (voluntary) placement, hospitals who took low numbers of students or the students who used a revised version of the manual. However, all current findings will be passed on to those overseeing the placement scheme, as part of its review and to ensure that it is as effective as possible in preparing WSoP students for professional life.

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Discovery of a kinase involvement in the ZIP7 mediated zinc wave

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Tamoxifen is the current gold standard treatment for most breast cancers but its success is limited by the onset of resistance to this anti-oestrogen. In order to study the mechanisms of resistance, a cell line called TamR has been developed to mimic the behaviour of tamoxifen resistant cells in the clinic. In this model, cell growth is mediated through activation of the EGFR/c-erbB2/MAPK signalling cascade¹. It has recently been shown that in TamR cells, there is an increase in intracellular zinc and in the expression of the zinc transporter ZIP7 when compared to the anti-oestrogen sensitive MCF7 cell line². Furthermore, in TamR cells, ZIP7 has been shown to be necessary in a phenomenon called the 'zinc wave'², which is described as a rise in intracellular zinc levels several minutes after an extracellular stimulus. The zinc wave originates from an intracellular compartment and is dependent on MAPK activation³. The release of zinc into the cytoplasm causes inhibition of phosphatases leading to activation of the tyrosine kinases EGFR, IGFR-1R and Src and the downstream signalling molecule MAPK in TamR cells. Hence, ZIP7 has been implicated as a target in breast cancer but the exact mechanism of action for this transporter is not yet clear. A human genome phosphoscreen has now shown several members of the ZIP subfamily of zinc transporters to have one or more phosphorylation sites suggesting that phosphorylation may be important in activation of the transporter function. ZIP7 contains two phosphorylation residues; one at serine 275 and one at serine 276. Therefore, this project aimed to (1) predict which kinases phosphorylate S275 and S276 using specialised computer software and (2) to investigate the temporal association of MAPK and other predicted kinases with the ZIP7 mediated zinc wave.

The ZIP7 protein sequence was input into two databases to generate predicted phosphorylation sites. The association of the predicted kinases with ZIP7 was investigated by western blot analysis of TamR cells immunoprecipitated with ZIP7. To create the zinc wave, cells were treated with 20µM zinc and 50µM zinc ionophore for 0 to 30 minutes. Western blots were quantified by normalising band intensity to ZIP7 using densitometry. Average densitometric data, from three separate experiments, was statistically analysed using ANOVA with Post Hoc tests to compare results to the non-treatment group.

Results found that ZIP7 was predicted to be phosphorylated by three kinases and these phosphorylation sites included S275 and/or S276. Visualisation of the immunoprecipitated samples probed with an antibody to kinase 1 showed a rise and fall in the level of association with ZIP7 over the time course. This increase in association was statistically significant at 5 minutes, the time of maximal association. A comparison of events during the zinc wave showed that the association of kinase 1 and ZIP7 peaks prior to the increase in activation of proteins by tyrosine phosphorylation. This is suggestive of a role for this kinase in the activation of the ZIP7 mediated zinc wave. The association of kinase 2 with ZIP7 during the ZIP7 mediated zinc wave was also observed from results. Although, statistical analysis of the average normalised data, did not find a significant difference over the time course of zinc treatment. This indicates that kinase 2 is not involved in activation of ZIP7. Investigation of kinase 3 showed no evidence of association of this kinase with ZIP7 despite the presence of ZIP7 bands demonstrating a successful immunoprecipitation process. These results show, for the first time, the association of 2 kinases with ZIP7 in cells. Furthermore, the association of kinase 1 with ZIP7 is enhanced in the correct time frame to suggest an involvement in the activation of the ZIP7 mediated zinc wave. Although this data doesn't conclusively prove whether this kinase phosphorylates ZIP7 on S275 and S276 as predicted by two independent databases, the results provide important information contributing to the currently unknown mechanism of action for zinc transporters such as ZIP7. A key role of this kinase in the activation of ZIP7 would have important implications for breast cancer treatment by facilitating easier targeting of ZIP7 in the clinic through the use of an appropriate inhibitor.

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Glutamate gliotoxicity in cerebral ischaemia-reperfusion injury

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Historically it was believed that the pathology of ischaemic stroke was a result of hypoxic neuronal death. It has become apparent that during periods of ischaemia, such as during stroke, glial cells, especially astrocytes are damaged and that glial cell death contributes to the overall pathology associated with stroke¹. Current treatment of stroke aims to reverse the ischaemia by reperfusion of the affected tissue area. Paradoxically however, reperfusion results in further cell death beyond that caused by ischaemia alone, termed ischaemia-reperfusion injury². Excessive release of L-glutamate is associated with cerebral ischaemia-reperfusion, caused by reversal of astrocytic glutamate transporters³. At high extracellular concentrations, L-glutamate is gliotoxic. Activation of the NMDA receptor has been suggested as a mechanism of L-glutamate toxicity in astrocytes⁴. This study intends to identify the role of glutamate in astrocytic cell death in response to ischaemia-reperfusion injury via assessing the role of the glutamate transporters and NMDA receptor.

An in vitro model of simulated ischaemia-reperfusion was developed using MOG-G-UVW human astrocytoma derived cells as a model of astrocytes. MOG-G-UVW cells were incubated in Modified Esumi's Buffer (deoxyglucose 10mM, K⁺ 12mM, pH 6.4), containing drug treatments (NMDA receptor inhibitor MK-801 (10µM; glutamate transporter inhibitors PDC (10µM) and ACBD (10µM)), in a hypoxic chamber with the air removed and flushed with N₂ gas in order to simulate ischaemic conditions. Cells were incubated at 37°C, at standard humidity, under simulated ischaemic conditions for 1 hour. Following 1 hour simulated ischaemia the cells were removed the hypoxic chamber and the Modified Esumi's Buffer replaced with Modified Esumi's Control Buffer (D-glucose 10mM, K⁺ 3.8mM, pH 7.4) to simulate reperfusion. Cells were incubated at 37°C, in an atmosphere of 5% CO₂ and 95% O₂, at standard humidity, under simulated reperfusion conditions for 1 hour. An L-glutamate concentration response was also performed. MOG-G-UVW cells were incubated with varying concentrations of L-glutamate (10nm to 10mM) at 37°C, in an atmosphere of 5% CO₂ and 95% O₂, at standard humidity for 24 hours MOG-G-UVW cells. The cell viability following simulated ischaemia-reperfusion and Lglutamate concentration response was measured using the MTS assay.

Inhibition of the NMDA receptor by MK-801 demonstrated no significant effect on cell viability compared to simulated ischaemia-reperfusion control when given at ischaemia or reperfusion. Inhibition of the glutamate transporters by PDC and ACBD demonstrated no significant effect on cell viability. L-glutamate demonstrated no toxicity to cells at all concentrations tested.

These findings suggest that in MOG-G-UVW astrocytoma cells, L-glutamate is not the cause of cell death following ischaemia-reperfusion. This study shows that the NMDA receptor and glutamate transporters are not involved in the mechanisms underlying cell death in simulated ischaemia-reperfusion. Furthermore MOG-G-UVW astrocytoma cells are not sensitive to L-glutamate toxicity. The findings of the present study differ to others reported in the literature and the cell type chosen in this study may be the reason for this confusion.

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Investigational synthesis of a novel co-drug for the treatment of psoriasis

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Topical drug delivery is the most popular approach for drug therapy in psoriasis. Delivery through the skin offers advantages over other methods, by enabling localised delivery of drugs to the target site, reducing systemic adverse effects. The co-drug strategy is a method of improving topical drug delivery by modulating unfavourable physicochemical properties of drugs and enhancing the delivery of both constituents. This strategy offers an attractive approach for anti-psoriatic therapy. A co-drug is a combination of two therapeutically active agents joined by a covalent linker, to have a synergistic pharmacological effect on a diseased state.¹ The aim of this research was to probe the co-drug concept further and synthesise a novel co-drug for the treatment of psoriasis. The synthesis of this co-drug was not as simple as predicted. In conclusion, the synthesis of a novel was not successful in the timescale available but the insights gained will aid further investigation.

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Impact of the reclassification of oral sumatriptan on the prescribing trends of oral triptans in Wales

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In May 2006 the legal status of sumatriptan in the United Kingdom (UK) was changed from prescription only medicine (POM) to pharmacy medicine (P) allowing it to be sold from pharmacies without a prescription under the supervision of a pharmacist. Sumatriptan belongs to the triptan therapeutic class and these are 5-HT agonists used in the treatment of acute migraine. Migraine is a primary headache disorder which is a major global health problem¹. It is able to cause significant morbidity in sufferers through an intense headache. Approximately 1 in 7 of the UK's population are thought to suffer from the condition which is believed to cost the UK economy £2.25 billion per annum². Imigran Recovery is currently the only triptan to have a marketing authorisation in the UK as a P medicine and contains two oral tablets of sumatriptan succinate 50mg. The aim was to establish the trend in prescribing of triptans before and after the reclassification of oral sumatriptan from POM to P.

To establish trends in the prescribing of triptans a retrospective analysis was undertaken of prescribing data in Wales for all triptans. Prescription data from June 1st 2003 to May 31st 2008 was analysed to provide data from a three-year period before the reclassification of sumatriptan and a two-year period after. A retrospective analysis of sales data in Wales of Imigran Recovery from June 1st 2006 to May 31st 2008 was performed to observe any trends in the immediate two years following the availability of sumatriptan as a P medicine. The North East of England was selected as a comparator region to Wales due to its similar levels of deprivation and the regions sell-in data was analysed to compare with Wales. The South East of England was selected as a region of contrast due to its lower levels of deprivation when compared with Wales. To analyse the effect of the reclassification on NHS expenditure on triptans, a retrospective analysis of expenditure data from Wales was performed using data from April 1st 2004 to March 31st 2008 to provide trends from the two-year period before reclassification and the two-year period after. To explore the influence of deprivation on the data the Welsh Index for Multiple Deprivation 2005 was used to rank each of the 22 LHBs in Wales in order of deprivation. Wilcoxon signed ranks test was used to test for the statistical significance of the results and data was analysed using Microsoft Excel 2000 and SPSS version 16.

The number of items of sumatriptan dispensed in Wales increased over the five-year study period by 31.59% (12.16 items per 1000 population to 16.00 items per 1000 population). The number of packs of Imigran Recovery sold in Wales increased over the two-year study period by 7.48% (1.07 items per 1000 population to 1.15 items per 1000 population). NHS expenditure on all triptans decreased between 2004/05 and 2007/08 by 6.74% while expenditure on only sumatriptan decreased by 25.30% over the same period. Imigran Recovery sales were 28.48% higher in the least deprived LHBs in Wales compared to the most deprived over the two years studied. The number of items of sumatriptan prescribed per 1000 population was 69.12% higher in the least deprived LHBs compared to the most deprived.

The reclassification of sumatriptan from POM to P had no apparent impact on the prescribing trend of sumatriptan in Wales as the number of items per 1000 dispensed continued to increase following the reclassification. The number of items per 1000 population of sumatriptan prescribed was higher in the least deprived LHBs in Wales. Sales of Imigran Recovery increased over the two years following its launch. Sales of Imigran Recovery were higher in the least deprived LHBs in Wales as expected possibly due to the high pack price. The English regions chosen for comparison followed a similar trend as sales were higher in the South East of England when compared to Wales while sales in the North East of England were similar to those in Wales. The statistical significance of the results show that deprivation may have some association with trends in prescribing and sales of sumatriptan in Wales. Future study could involve the analysis of the trends over a longer period of time and in a larger population, possibly the UK as a whole.

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An attempt to improve Fedors' fragmental values used to determine solubility parameter

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Knowledge of the permeability of skin to chemicals is essential in assessing clinical effectiveness and toxicity. Reliable predictive methods would reduce the need for experimental determinations. Hildebrand solubility parameters (δ) have been used as one of the predictive properties, and are calculated by the square root of the enthalpy of vaporization (ΔH_v) divided by the molar volume (V_m) of a compound.¹ Fedors proposed values for these properties for various functional groups and suggested that δ for a molecule could be estimated an additive method. These fragmental values were listed for 88 functional groups and were applicable 25°C and 1 atmosphere pressure.

The primary aim of this investigation was to improve upon those fragmental values predicted by Fedors. Experimental values for the enthalpy of vaporization and molar volume were obtained for 310 compounds containing various functional groups. The functional group contributions to ΔH_v and V_m were estimate by regression analyses. After removal of unreliable values 28 functional group contributions for both ΔH_v and V_m were obtained. Use of these values gave more accurate and precise predictions of δ than those reported by Fedors.

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A comparison of drug stability in ambulatory devices

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Ambulatory devices allow patients to receive continuous drug therapy in any environment. They are intravenous devices attached to a patient through a catheter, which they can carry with them, enabling the patients to maintain a normal lifestyle. In order to use a device such as this, thorough patient training is required to ensure correct use, whilst drug stability is an important issue in all formulated drug products. Instabilities can lead to an increase or decrease in drug concentration which can lead to patients receiving either a toxic or sub-therapeutic doses. It can also lead to the presence of degradation products which could be harmful to the patient. There is currently little known about the stability of drugs when in different ambulatory devices, so for this reason it was investigated.

Four different ambulatory devices, an ANAPA®, an Eclipse®, an EVA bag and a syringe were tested. When activated, the ANAPA® device generates carbon dioxide in its top chamber which is then used to create pressure to push the drug solution in the bottom chamber through to the patient¹. The Eclipse® device is made from plasticised PVC which is elastomeric and expands on filling and then contracts to push the solution through² on use. EVA bags and syringes are not technically ambulatory devices, but as they can be used for the same purpose, they were treated as such for this study. The devices were filled with either a mixture of fentanyl 2µg/ml and bupivacaine hydrochloride 1mg/ml (0.1%) as a drug solution, or water, and then tested daily for particulate matter, turbidity, pH, drug concentration, and dissolved oxygen saturation. They were also tested in a variety of conditions, including exposure to light, in the dark, high and low temperature, and additionally with different lengths of time.

It was found that solutions stored in the Eclipse® device showed an increase in pH, which was thought to be due to the plasticiser (DEHP) in the device walls leaching into the solution. In practice this would lead to human exposure to the plasticiser which could have adverse effects, and so renders this device unsuitable for storage of solutions in which this would happen³. The concentration of fentanyl also decreased significantly when stored in the Eclipse® device in all conditions, this was thought to be due to the drug adsorbing to the wall of the device, a process which has been reported previously with PVC with numerous drugs⁴. When water was stored in an activated ANAPA® device for 9 days, a significant decrease in pH was observed which was due to carbon dioxide escaping from the top chamber and coming into contact with the solution, this change however, was not observed when the device was filled with drug solution, which could be due to solubility differences. This device is therefore not suitable for storage of pH sensitive solutions. The EVA bag was found to be oxygen permeable after an increase in dissolved oxygen saturation was observed in solution stored in it, thus making it unsuitable for storage of solutions susceptible to oxidative degradation.

As the syringe was the only device in which no changes were observed, this is the most suitable device for storage of drug solutions, as minimal degradation would occur, however, as a syringe is not actually an designed as ambulatory device, it is unlikely it would be used as one in practice.

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Vaccine wastage in GP practices

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Vaccine wastage is defined as the supply of vaccine that is never administered. Currently, more than £300 million is spent on purchasing vaccines each year in the U.K. As new vaccines are introduced into immunisation schedules the scale of vaccine wastage is likely to increase¹. Vaccine wastage has the potential to be costly and damaging to immunisation programmes. Little data is available on the nature and extent of vaccine wastage in Wales, hence the need for further investigation. Primary aims were to establish principles of good practice in vaccine management and to develop strategies to reduce wastage.

For a preliminary overview of wastage in South Wales practices, 10 nurses were interviewed from four different LHBs. The main themes explored were; vaccine incidents, vaccine management training and knowledge and compliance with guidelines.

Instances of vaccine wastage had occurred at nine out of 10 of the practices. The major cause of wastage was attributable to vaccines passing their expiry dates as a consequence of over-ordering. In each instance no record of wastage was documented and contrary to advice, wastage incidents were not reported to LHBs or immunisation co-ordinators. Overall it appeared that most practices took preventive measures to ensure vaccines were not wasted but only after a previous incident had occurred. Drawing attention to particular issues encouraged participants to re-evaluate current practice.

Responses suggested that management of vaccine wastage did not feature high on the list of priorities at GP practices. Many causes of vaccine wastage could be limited by adopting good management practices. Implementation of both routine reporting and monitoring systems in all GP practices across Wales would therefore be beneficial to minimise wastage.

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The effect of the 'friendly bacteria' *Lactobacillus casei* Shirota on the isolated ileum

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Widespread advertising campaigns have made probiotic products more prominent in recent times and the annual market is now worth over £135 million in the UK alone¹. Despite this their mechanisms of action remain largely unknown. Previous theses investigated the effects of the popular probiotic drink, Yakult, on the isolated ileum. It was discovered that Yakult caused an initial relaxation of the ileum followed by a recovery to over 100% of its original contraction². It was speculated that the recovery was due to the release of trace amines (TAs) by *Lactobacillus casei* Shirota (LcS), the live bacteria in Yakult². TAs are a type of low molecular weight biogenic amines that are present endogenously in nanomolar concentrations³. They were originally thought to act as neuromodulators, their actions increasing or decreasing uptake of other amines. Recent discoveries of TA specific receptors, named trace amine associated receptors (TAARs) have suggested that they might have a role as neurotransmitters³. Tyramine and β -phenylethylamine are the most studied TAs. They are metabolised *in vivo* by monoamine oxidase enzymes³. The aim of this study was to explore how a pure culture of LcS affected the contraction of the isolated ileum.

LcS was extracted and isolated from fresh Yakult and grown overnight on a shaking plate at 37°C in MRS broth. Optical density readings of the broth were taken in order and compared to a calibration curve in order to obtain bacterial concentration of 1×10^9 viable cells per ml. Sections of ileum, 2cm in length, were suspended in Krebs solution in organ baths and electrically stimulated. 1×10^9 viable cells of LcS were added to the organ bath and incubated for two hours. Baseline tension and twitch height responses were measured. Further experiments were performed with LcS plus precursors (tyrosine, phenylalanine and pyridoxal phosphate – substrates for TA synthesis) and LcS plus precursors and pargyline (a non-selective MAOI) incubating in the organ bath for two hours. Neat Yakult and Yakult plus precursors and pargyline experiments were also performed over 120 minutes. Cumulative concentration response curves to tyramine were performed prior to all LcS or Yakult experiments.

Tyramine caused a decreased twitch height: a concentration of 3×10^{-3} M caused a mean decrease to 73.45% ($\pm 1.98\%$ SEM) of its original twitch height. A biphasic response was seen with regards to tyramine's effect baseline tension: maximum baseline tension was seen at 10^{-3} M followed by a relaxation to 69.34% ($\pm 3.48\%$) at 3×10^{-3} M. Neat LcS also caused a decreased twitch height; significant differences were found between its value of 80.70% ($\pm 4.69\%$) and a control (no bacteria, precursors or pargyline) after 120 minutes using a two-way ANOVA followed by a post hoc Bonferroni test ($P < 0.05$). A further decrease to 78.65% ($\pm 5.13\%$) was seen with the addition of the precursors. LcS plus precursors and pargyline caused a significant increase in baseline tension, similarly to tyramine. A maximum tension of 143.08% ($\pm 6.41\%$) was reached after 105 minutes. This was value was statistically significant in comparison with a control (102.89% $\pm 1.26\%$) and neat LcS and LcS plus precursors at the same time point. Yakult and Yakult plus precursors and pargyline caused an initial relaxation of the twitch height of the ileum, to 11.70% ($\pm 4.75\%$) and 22.38% ($\pm 6.81\%$) respectively, before recovering to almost 100% of their starting heights. Significant differences between these experiments and a control were seen during the initial relaxation phase. Both Yakult experiments also caused an initial decrease in baseline tension; however the precursors and pargyline caused a larger recovery than neat Yakult (109.52% $\pm 9.89\%$ compared to 85.67% $\pm 9.58\%$).

The results suggest that LcS synthesises TAs which cause a contractile effect. LcS and Tyramine experiment results show similar twitch height and baseline tension trends. The addition of precursors caused increased responses, suggesting that the extra availability of substrates led to extra TA synthesis. Pargyline addition caused a large increase in baseline tension implying that the TAs synthesised by LcS had a prolonged exposure to the ileum to cause contractions due to MAO inhibition. TAs released by LcS in Yakult cause a lesser effect than those synthesised by pure cultures, hinting that the constituents of the probiotic drink may be masking the effects of TAs. This investigation has found that probiotics produce a TAs and cause a contractile response in the ileum. With continued research there is potential for probiotics to be used clinically in the future in the treatment of IBS symptoms such as bloating, cramping and constipation.

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Rationalising equilibrium data analysis for EDGMA/MAA molecularly imprinted systems

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Molecular imprinted polymers (MIPs) are crosslinked polymeric materials that act as artificial receptors¹. They are formed through co-polymerisation of functional and cross linkable monomers in the presence of a target or template molecule¹. MIP performance is commonly analysed through batch rebinding assay. This rebinding data can be displayed in a number of different ways, e.g. bound vs. free plot and Scatchard plot. The study is aimed to re-interpret reported MIPs equilibrium binding assay data as a standard binding isotherm thus allowing inter-polymers comparison to be made.

Literature review of papers published on MIPs that were made using EDGMA, MAA and AIBN via monolith polymerisation was done. The binding data was normalised into a standard unit of nmol/mg for bound and μM for free and plotted as a bound vs. free isotherm. MIPs data then entered into GraphPad software to estimate the B_{max} and K_d values. Relationships were sought between a number of variables (synthetic temperature, solvent, template hydrogen bonding ability and template molecular weight) and MIPs performance.

Although all the polymers were prepared in a similar manner, they do not behave in the same way. Each has its own binding capacity and affinity. UV initiated MIPs prepared at 0°C showed better binding capacity than polymers prepared at 60°C . A strong relationship was observed between affinity and decreasing solvent polarity, and capacity and increasing solvent polarity. However a relationship between hydrogen bond donor/acceptor and K_d and B_{max} was not observed. Furthermore, there was no obvious relationship observed between molecular weight and binding capacity or molecular weight and binding affinity.

From an extensive literature review of papers published between 2004 to 2008, only 47 polymer evaluation systems were reported in sufficient detail so as to allow data normalisation. The key limitation to the study was under-reporting of equilibrium binding data which made data normalisation impossible. For the first time this study has comprehensively demonstrated a systematic comparison of MIP performance and in doing so has provided a route to rationalise form and function.

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Expediting patient access to medicines: Are companies achieving simultaneous submission and regulatory approvals in ICH countries?

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The pharmaceutical industry has a global market size in excess of \$600 billion¹. The industry has in recent times been the centre of much focus as its productivity levels have declined² whilst the research and development cost have continued to rise³. As the figures for drug development continue to escalate, the sustainability of the current development model is being questioned and the pharmaceutical companies are therefore re-evaluating their development strategy with focus on implementing a global development programme so as to enable them to submit simultaneously in a number of regulatory authorities. Regulatory authorities' role is to safeguard public health by only allowing medicines that are safe and effective to be approved whilst also ensuring that the drug approval process is carried out in a timely manner. However, pharmaceutical companies can also influence how fast a drug reaches the patient as the submission strategies used in the submission of a dossier to the various jurisdictions around the world create a time lag. The objective of this study is to review approvals of new active substances (NASs) by US FDA (Food and Drug Administration), EU EMEA (European Medicines Agency), Australian TGA (Therapeutic Goods Administration), Canadian TPD (Therapeutic Products Directorate), Japanese PMDA (Pharmaceuticals and Medical Devices Agency) and Swissmedic between 1997-2008, in order to determine whether the harmonisation of technical guidelines (ICH) has enabled simultaneous submissions and approvals in these markets.

Data were analysed for 731 NASs that were approved by the six agencies of which 48 were approved by all six. Submission and approval dates, approval route, company size and therapy area were analysed. whilst In addition actual submission patterns were compared to stated strategies for 12 companies in a 2006 survey done by CMR international on company filing strategies. Approval times have in the last decade improved across all the six agencies, with the FDA having the shortest approval time of 237 days in the year 2007. The EMEA improved on its approval times in the year 2003-2008. Expedited reviews products are approved much faster than standard reviews. The FDA has the fastest approval times for expedited reviews at 180 days. Anti-infectives for systemic use, Cancer agents have in the past decade been approved in the highest numbers. No major differences were seen between major and other companies for the FDA, EMEA, Swissmedic, TGA and Canadian TPD. However, Japanese PMDA seem to approve products from other companies much faster than for Major companies.

For the NASs approved by all agencies, the median time to submission ranged from 0 (FDA) to 907 days (PMDA) in a pattern similar to the reported strategy. Overall, the regulatory approval time has become more consistent, most noticeably by the Australian TGA and the EMEA who's adopted time frames for which the drug reviewing process is expected to take has resulted in this consistency. Variability related to therapy area and approval route were predominantly seen by the FDA, Swissmedic, PMDA and Canadian TPD.

Against a background of the ICH initiative which was started in 1992 with the aim of harmonizing technical guidelines for dossier applications between the FDA, EMEA and Japanese PMDA, it should have been easier for companies to simultaneously submit their dossier applications to these three regions. submission patterns however suggest that companies are adopting a three-tier strategy, with simultaneous submission to the FDA and EMEA in the first 30 days (median), followed by submission to the TGA, TPD and Swissmedic 90-180 days (median) later and finally to the PMDA where most submission are done after one and a half to two years later.

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An evaluation of the prescribing practice and management of chronic obstructive pulmonary disease at Llanrumney Medical Centre

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Chronic Obstructive Pulmonary Disease (COPD) is an umbrella term used to describe long-term conditions that cause airflow obstruction in the lungs. The airflow obstruction is not fully reversible and is usually progressive with time. Clinical symptoms include a chronic cough, shortness of breath, respiratory tract infections and complications such as depression. COPD is caused by long term damage to the lungs most frequently by smoking. Low socioeconomic status is also a risk factor. COPD is a leading cause of morbidity and mortality. The prevalence of COPD and the prevalence of the main risk factor, smoking, are particularly high in the deprived ward of Llanrumney.¹ There are three principal evidence-based guidelines published for COPD whose recommendations are currently used worldwide.^{2,3,4} In addition, a COPD Quality and Outcomes Framework (QOF) exists which is the system used to pay UK General Practitioners based on the quality of care given. The aim was to evaluate the prescribing practice and management of COPD at Llanrumney Medical Centre.

The standardized data set collected for the evaluation was developed using evidence-based clinical guidelines and QOF indicators, and from discussion with Llanrumney Medical Centre. There were seven sections of the data collection form, these were; patient demographics, nutritional status, smoking cessation advice and therapy, immunizations, airflow obstruction severity, inhaler technique and prescribed treatment for COPD and depression. Data was collected for 7 patients during the pilot of the data collection form. Adjustments were made to increase the space available on the form. Data was collected for patients on the COPD register by researching retrospective electronic case notes using the NHS Wales Vision system. There was one primary researcher. SPSS Version 16 was used analyse the data.

The number of patients identified from the COPD register and included in the evaluation was 205. The current mean age of patients was 71 years (SD±10.46) (n=205). The age when added to the COPD register ranged from 33 to 94 years (n=194). The male to female ratio was 50.7%: 49.3% (n=205). The percentage of current smokers, ex-smokers and never smokers was 38.5%, 53.7% and 7.8% respectively (n=203). COPD diagnosis had been confirmed with a spirometer in 31.4% of patients, of which only 4.6% was reversibility testing (n=194). A spirometry test had been performed by 131 patients within the past 15 months to monitor the disease progression (n=205). Smoking cessation advice had been given to 83.5% of current smokers within the past 15 months (n=79). Smoking cessation therapy had been prescribed for 32.9% of patients in the past 12 months and the number of smoking cessation therapy prescriptions issued ranged from one to five over the past 12 months (n=26). The frequency of documented dietary advice given within the last 15 months was very poor for patients underweight, overweight, obese or very obese (n=199). A pneumococcal vaccine had been administered to 69% of patients and 80% of patients had received an influenza vaccine in the past 12 months (n=205). Inhaler technique had been checked in 49% of patients within the last 15 months (n=202). Forty-four patients were prescribed an anti-depressant in the past 12 months (n=205). The most frequently prescribed drugs were salbutamol and tiotropium for stable COPD management. It was found that 90 patients current treatment did not follow the recommended prescribing practice (n=116).

This evaluation based in a primary care setting in a deprived area was successful in identifying a number of concerns in the current practice of Llanrumney Medical Centre. In particular, improvements are needed to increase the frequency of dietary advice given, inhaler techniques reviewed and reversibility testing performed to confirm COPD diagnosis. The findings suggest that the awareness of up-to-date prescribing recommendations may need to be reviewed. However, as symptoms are variable in COPD the clinician must use their knowledge and experience to determine the best course of treatment for each individual patient.

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Haptics in drug design

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Rational drug design is a focussed approach, which uses information about the structure of a protein target or one of its natural ligands to identify or create candidate drugs. One of the most popular rational drug design protocols is docking and scoring. However, rational drug design is primarily a pattern recognition problem, an area where humans often flourish, whilst algorithms customarily have difficulty in encapsulating such concepts. Even with the improving accuracy of docking simulations, algorithms alone cannot account for every phase in a problem as complex as drug design. An innovative approach to improving molecular simulations is the application of haptics. Haptics (in this context) refers to the science of touch (tactile) sensation and control of virtual environments with computer applications. Enabling the modeller to interface with his/her working environment makes the modelling process more meaningful as it permits utilization of an invaluable asset—human intuition. To validate the methodology the biological EGFR kinase was selected. Perturbation of kinase function has been implicated in numerous disorders, including immunological, neurological, metabolic and infectious disease¹ and is particularly illustrious for its implications in cancer

A comparison of three docking programs (FlexX, Multifragment Search, and Plants³) and two different haptic devices was carried out. The haptic devices were supported by a tailor made software program called Zodiac⁴. In phase one fragment databases were created from known ligands which bind in the ATP binding site. In phase two, fragments were designed to optimise binding interactions in the allosteric C terminal of EGFR kinase. Results obtained from the simulations were re-scored using an independent scoring function called dock pki. The dock pki indicates the binding affinity of a particular suggested pose, and so the higher the value, the stronger the interaction, subsequently the better the pose.

Generally docking is better suited to handling larger ligands and isn't as effective when working with fragments. Results revealed that in terms of placement, the Plants algorithm performed the best compared to other docking algorithms programs. Overall however, the haptic device performed the best in terms of placement as it was user guided and involved the knowledge and intuition of the user during the docking simulation. On the contrary, in terms of scoring, the best dock pki does not always correlate with the best pose. This negative relationship is attributable to the fact that only one scoring function was used to assess all five methodologies, which isn't a particularly reliable method for selecting the best pose. We speculate that the use of more than one scoring function would improve the relationship between visual inspection and scoring. In addition, as previously mentioned, scoring functions have not previously been applied to data generated from haptic simulations

In conclusion haptic guided simulations perform equally as well as, if not better than computer simulations with docking algorithms. The haptic devices is a sophisticated tool allowing elaborate drug design and can be considered for use in conjunction with docking and scoring. This exciting field is sure to develop and will no doubt become an asset to the molecular modelling toolbox.

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Aripiprazole: 2-year outcomes in a retrospective naturalistic follow up study

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Schizophrenia is a chronic, debilitating, mental health disorder that occurs in approximately 1% of the adult population¹. Aripiprazole is a novel treatment for schizophrenia that acts as a partial agonist at D₂ and 5HT_{1A} receptors. The efficacy and tolerability of aripiprazole has been evaluated in randomised controlled trials (RCTs), however these studies may not reflect clinical practice. RCTs employ strict inclusion and exclusion criteria, and are often of short duration. Naturalistic studies focus on real life outcome measures, are more representative of practice, and can include longer term follow up. The aims of this naturalistic study are to investigate the clinical effectiveness, safety, and tolerability of aripiprazole in patients with schizophrenia and schizoaffective disorder. To analyse 2-year patient outcomes following aripiprazole treatment in an acute mental health trust.

Patients prescribed aripiprazole between July 2004 and January 2007 were included in the study, and data collected by retrospective case note review. Subjects were categorized either as those remaining on aripiprazole at 2 years, designated 'continuers', or individuals who stopped treatment 'discontinuers' Those previously treated with clozapine were identified and considered treatment resistant. The primary aim of this study was to evaluate the clinical effectiveness of aripiprazole to treat schizophrenia. Effectiveness takes into consideration the impact a drug has on controlling the variable symptoms of the illness, as well as its safety and tolerability. Thus, the decision to continue or discontinue treatment would reflect the combined evaluation of efficacy, safety, and tolerability by both the patient and the prescriber. Treatment discontinuation was therefore the selected primary outcome measure.

Out of the total 159 patients that had been prescribed aripiprazole, ninety one patients were eligible for inclusion in the study. At 2 years, 26 (29%) patients remained on treatment and 65 (71%) discontinued. The main reasons for aripiprazole initiation were ineffectiveness and side effects of previous antipsychotic. The main reasons for treatment discontinuation were insufficient efficacy and patient reported non-adherence. The highest rates of treatment discontinuation were observed to be immediately after treatment initiation (in the first 3-6months), with the lowest rates between 12-24 months. Age, gender, ethnicity, dose of aripiprazole and concomitant medication did not appear to affect discontinuation or continuation with aripiprazole. However, outpatient status at initiation was significantly associated with outcome ($P=0.0363$). Patients who have previously had two or more antipsychotic treatment failures were no more likely to discontinue treatment than those who had not. Moreover, Patients previously treated with clozapine (and considered treatment resistant) were also no more likely to discontinue aripiprazole than those who were clozapine naïve; dissimilar to the widely accepted concept that clozapine is the only effective treatment choice in treatment resistant patients.

Despite the limitations of the naturalistic methodology, it appears that aripiprazole is relatively well tolerated and effective in clinical practice The findings in this study in regard to the discontinuation rate are not directly comparable to the large effectiveness trials (CATIE, CUtLASS, EUFEST, SOHO and BETA) as this is the only study of its nature that has reported outcomes with aripiprazole at 2 years. However, the discontinuation or continuation rate can be compared with these studies at their respective endpoints. The 41% continuation rate observed at 6 months is comparable to that seen with aripiprazole in the study by Deslandes², (2008) and with risperidone, quetiapine, perphenazine and ziprasidone in the CATIE³ study. A 68% continuation rate at 8 weeks is also comparable to haloperidol in the BETA⁴ study. Additionally the findings in this study suggest that there is scope for a trial of aripiprazole for patients with treatment resistant schizophrenia and is an invitation for further research in this area.

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Expression of clathrin-dependent and -independent endocytotic proteins in an Alzheimer's disease model

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Alzheimer's disease (AD) is a progressive neurodegenerative condition characterized by deterioration in memory and cognition¹. Amyloid beta (A β) deposition is a major pathological feature of AD and is formed following two sequential cleavages (by β - and γ -secretases) of a transmembrane protein, amyloid precursor protein (APP). Endocytosis of APP may occur via two pathways, the clathrin-dependent route, involving clathrin-coated pits, and the clathrin-independent route involving lipid rafts and caveolar pathways. Endocytic changes occur in AD that may affect APP turnover and constitutive A β generation². The model of AD used here expresses the double Swedish mutation of APP located immediately adjacent to the β -secretase cleavage site, resulting in over-expression of APP, along with robust pathological changes resembling those seen in AD³. My project was aimed at investigating the expression of several important endocytic proteins involved in clathrin-dependent and independent endocytosis in cell lysates from this model and attempting to determine whether the APP over-expression is associated with changes in the expression of endocytic proteins.

Western Blotting was performed to determine the expression of some key endocytic proteins in transgenic and genetically unmodified cortex lysates. Subsequent quantification of the immunoblots was performed via densitometric analysis to compare the expression of the proteins in the transgenic model with their unmodified equivalents. β -actin was used as a standard loading control for the immunoblots, and the densitometry analysis of the observed bands was normalised to β actin levels. Statistical analysis was performed using unpaired Student's *t*-tests.

Results from Western Blotting confirmed a ~10 fold increase in APP expression in the transgenic model compared to unmodified equivalents, with the mature form of APP detected at a molecular mass of ~111kDa. The mature form of the BACE-1 (β -secretase) protein migrated at ~71kDa and its expression also increased markedly in the transgenic model, whereas no change was observed in the expression of the homologous BACE-2 associated protein. Clathrin was detected at ~188kDa, and protein levels were significantly increased in the transgenic model compared to the unmodified equivalents. Flotillin and PICALM proteins were also detected in all cortex lysates, at ~50kDa and ~71kDa, respectively, with a slight increase in protein levels again observed in the transgenic model. When probed with anti-caveolin-1 and anti-caveolin-3 antibodies, both immunoblots displayed bands at the expected molecular masses of ~23kDa for caveolin-1 and ~24kDa for caveolin-3, with densitometry analysis revealing no significant change in expression levels of proteins between the transgenic and the unmodified equivalent models.

The proteins studied were chosen due to their role or potential role in the endocytic processing of APP. APP over-expression in the transgenic model was confirmed, and is likely to be closely associated with the increase in BACE-1 levels seen here. BACE-1 is a key enzyme in the processing of APP to form A β , and is associated with both the endocytic pathways mentioned. My studies suggest a rise in BACE-1 levels may be a result of amyloid pathology rather than the result of cell death, as major neuronal loss is absent in the transgenic model. Clathrin is a key protein involved in the internalisation of APP by clathrin-coated vesicles, and PICALM also plays a role in clathrin-mediated endocytosis⁴. The increased levels of both proteins suggest they are in close association with the APP over-expression in the transgenic model. Flotillin also has a proposed role in APP endocytic processing, acting as a recruiter of APP for lipid rafts, and the rise in flotillin expression may reflect this. The fact that caveolin-1 protein expression was similar in both the transgenic and unmodified models suggests that the caveolar pathway may not be associated with APP over-expression in this transgenic model of AD. These results are in contrast to the clathrin-dependent pathway which is possibly directly associated to the APP over-expression. My results support the changes in endocytosis described in the literature in AD², and suggest that these proteins may be important for the aetiology of the disease.

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Evaluation of the types of 'Ceramide' included in skin-care products and meta-analysis of published data on the effects of topical application of 'Ceramides' containing products to normal and damaged skin

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Ceramides are the most abundant lipids present in the stratum corneum. Ceramide deficiencies and abnormalities have long been linked to a wide range of skin problems and genetic conditions such as atopic dermatitis, psoriasis, skin ageing and Gaucher's disease and topical ceramide therapy has been employed to treat these issues. Many extravagant claims have been made regarding the effects of topical applications containing ceramides and there is much speculation as to whether these claims are fact or fiction. Numerous types of ceramide have been used, ranging in composition from nature identical and naturally occurring to entirely synthetic derivatives. It has been questioned whether topical application of 'ceramides' can be enough a significant effect in treatment of skin conditions and healthy skin. Much has also been speculated regarding how much 'ceramide' is actually incorporated into popular consumer products. Concerns have been raised with respect to both long and short term usage of such topical applications and health concerns have been raised in the past in reference to what and how commercial 'ceramides' are sourced. The aim of this investigation was to evaluate what claims were being made, what materials were being used in current skin care compositions and how these were produced. In addition the question of whether 'adding back' was effective was investigated, as well as considering the safety of these applications.

Claims, ranging from the extravagant to the scientifically justifiable, currently being made by cosmetic and drug companies were initially investigated through a keyword search using Google™. This investigation was then expanded into other larger scientific search engines such as WIPO and Freepatentsonline. An investigation into what types of ceramides had been previously and currently used in topical applications was carried out using the same method of keyword search. This was followed by a search and evaluation of specific patent documents. The annual number of ceramide related patents was also evaluated.

Results of the claim search indicated that there was a solid basis for the claims made by certain drug companies but that these somewhat become overstretched when involved in the cosmetic area. Results also showed that ceramide based compositions were now almost entirely produced via chemical synthesis. Results of the patent investigation demonstrated that the area of ceramide technology is still in growth phase and is yet to see a decline in applications for various types of ceramide compositions.

While the use of natural and skin identical ceramides pose potential risks and high manufacture cost, synthetic ceramides or pseudoceramides have been developed as ceramide alternatives. In vivo studies of pseudoceramides in both experimental models and preliminary human studies have demonstrated that topical Ceramide-containing lipid mixtures can improve barrier function.¹ Research and patent papers have presented novel synthetic ceramide derivatives that have shown good drug delivery due to their stereochemistry as well as other crucial factors such as lipophilicity. Pseudoceramides have shown to be effective in delivery in addition to incorporating into the lipid layer and improving abnormalities being used mainly in ancillary treatment.² Extensive safety studies into pseudoceramides have been conducted including oral dosing as well as eye administration both demonstrating no evidence of irritation, sensitivity or toxicity. The amount considered to be suitable for topical application ranges from 0.001% - 25%, most preferably 0.01% - 2% by weight of the composition. This investigation has concluded that the concentrations used in the current market for topical treatments have been shown to be safe and effective in various ancillary treatments as well as some mainstream treatments and cosmetic treatments.

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The effect of tyramine on isolated atria

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The mode of actions of trace amines have been traditionally referred as sympathomimetic-like. In the cardiovascular system, a lot of studies suggested that the effect of trace amines (e.g. tyramine) was predominately indirect.¹ Tyramine could either trigger the efflux of catecholamines from the sympathetic nerve endings or inhibit the reuptake of catecholamines, hence triggered positive inotropic effects in the atria. After the discovery of trace amine associated receptors (TAAR),² it is believed that trace amines could stimulate TAAR directly to produce their effects. Recent studies on trace amines in isolated ileum showed that a wide range of trace amines could produce a biphasic response (contraction followed by relaxation). This effect was believed to be non-adrenergic non-cholinergic but could be influenced by ketanserin (a 5-HT₂ antagonist)³

The cumulative effects of tyramine alone and with different β -blockers (i.e. timolol and propranolol) or 5-HT₂ antagonist (i.e. ketanserin) were recorded in atria stimulated with an electrical field. Isoprenaline was used as a control to compare the effect of tyramine by examining the right-ward shifting of the concentration response curves using dose-ratio calculation. Differences between tyramine alone and with antagonists were identified by Student t-tests.

Results showed that the administration of timolol could reduce the maximum responses of tyramine and isoprenaline significantly. The shifting of the concentration response curves in the presence of timolol with tyramine or isoprenaline was significant. The shifts were 23-fold (pEC₃₀) and 26-fold (pEC₃₀) to the right respectively. Ketanserin did not reduce the maximum response of tyramine and it showed no shifting of the concentration response curve to the right.

In conclusion, this thesis proved that part of the effect of tyramine was β_1 -adrenoceptor mediated. It also shows that the involvement of α_1 -adrenoceptors is minimal. However, the blockade of β -adrenoceptors alone could not abolish the effect of tyramine suggesting other mechanisms or a direct role of tyramine could be involved (e.g. α_1 -adrenoceptor mediated, TAAR etc). Results showed that 5-HT₂ receptors did not affect the response of tyramine which does not match the findings from previous studies done on isolated ileum. Reduction of the maximum responses in the presence of competitive inhibitors was observed and discussed in this thesis.

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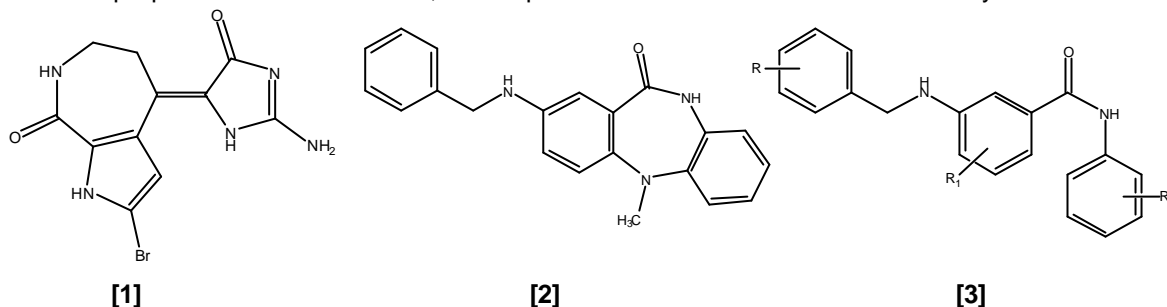
Design and synthesis of novel inhibitors of cyclin-dependent kinases (CDKs) as anti-proliferative agents

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Deregulation of the normal cellular mechanisms, such as those controlling cell cycle progression or DNA damage contribute to the development of neoplasias¹. CDKs are serine/threonine kinases which require association with a cyclin regulatory subunit for activity. CDKs regulate entry and progression through the cell cycle. Whereas healthy cells are able to stop at pre-determined stages of the cell cycle, cancerous cells lack this restraint due to loss of checkpoint integrity². The analysis of human tumours has demonstrated that cell-cycle regulators are frequently mutated in human neoplasias. In principle, CDK inhibition could recapture cell cycle checkpoints and reduce a malignant cells ability to proliferate and may even induce apoptosis.

Hymenialdisine (HD) [1] is a natural product isolated from various marine sponges. It competes with ATP to inhibit a number of CDKs at nanomolar concentrations³. Previous work of designing HD analogues led to the discovery of the dibenzo-1,4-diazepan-5-one derivative [2] which showed good anti-proliferative activity in cancer cell lines. However, this compound was difficult to synthesise. This project was concerned with the synthesis of the proposed 3-(benzylamino)-N-phenylbenzamide analogue [3] which was anticipated to have similar properties to the dibenzo-1,4-diazepan-5-one derivative but be easier to synthesise.



The most successful method employed for the preparation of this compound involved the synthesis of 3-(4-nitrobenzylideneamino)benzoic acid by reaction between 3-aminobenzoic acid and 4-nitrobenzaldehyde in THF. The nitro group introduced at the R substituent position was found to be beneficial in improving the yield of the compound and when reduced to the amino group produced favourable interactions in the ATP binding site of CDK2. The resulting 3-(4-nitrobenzylideneamino)benzoic acid was dissolved in THF with EDCI and DMAP to facilitate reaction with aniline to produce 3-(4-nitrobenzylideneamino)-N-phenylbenzamide. Although the reaction was successful in the addition of aniline, the imine bond proved to be unstable in solution and was cleaved. The resulting 3-amino-N-phenylbenzamide fragment was reacted again with 4-nitrobenzaldehyde and hydrogenated at for 1.5 hours at 30psi over a 10% palladium on carbon catalyst. ¹H-NMR indicated that the final compound 3-(4-aminobenzylamino)-N-phenylbenzamide has been successfully synthesised but was impure.

Repeating the synthesis on a larger scale would allow easier purification of the compound and produce sufficient material to undertake biological testing on cancer cell lines.

Molecular modelling revealed that the introduction of substituents at R2 could also produce favourable interactions at the ATP binding site and therefore potentially improve binding affinity.

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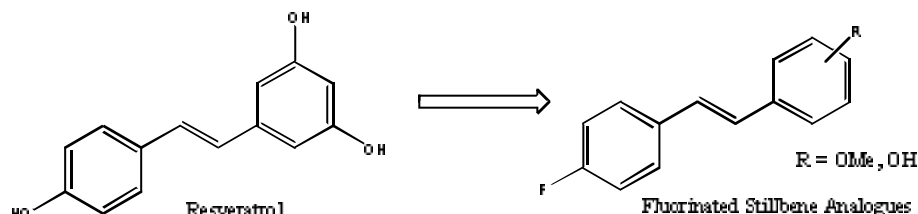
The synthesis and anti-tumour evaluation of fluorinated stillbenes

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The major problem currently surrounding cancer therapies' regarding their non-selectivity and resistance has led to greater interest in the chemo-preventative actions provided by naturally occurring molecules such as Resveratrol. Resveratrol, chemically known as trans-3, 5, 4'-trihydroxystilbene is a polyphenolic phytoalexin that has been shown to have many beneficial biological properties including being the cardio-protective constituent of red wine. Data from previous literature has proposed that Resveratrol's chemo-preventative actions are produced by effects on all three stages of carcinogenesis, through modulation of a variety of cell signalling pathways.¹ In this study we embark on the recent finding that Resveratrol can affect tumour-growth through inhibition of a specific pathway known as the Wnt signalling pathway. The Wnt signalling pathway is associated with the accumulation of a cytoplasmic protein known as β -catenin, which is a transcription activator for genes that control cell growth and differentiation.² Chronic activation of this pathway through aberrant activity has been shown to be an important feature in the drive of uncontrolled growth and survival of cancer cells and is known to be activated in over 85% of colon cancers.²

The main problem with Resveratrol due to extensive metabolism, is its low bioavailability and this as a consequence reduces its opportunity to be used within a clinical setting.³ In this study we expand on the results that more lipophilic analogues of Resveratrol have been found to have enhanced anti-proliferative and Wnt inhibitory actions.⁴ In this context, a small library of trans-fluorinated Resveratrol analogues were synthesised utilising the core stilbene pharmacophore, containing a variety of methoxy and hydroxyl substitute groups. A fluorine molecule was used as a substitute for the 4'-phenol group, in an attempt to prevent phase II metabolism and to increase lipophilicity. The use of a fluorine molecule allows these analogues to also have the potential to be used as PET imaging agents. The synthesis of the fluorinated analogues, initially involved the production of a 4-fluoro phosphonate ester intermediate using the Michaelis Arbusov Rearrangement. This phosphonate intermediate was subsequently reacted with a variety of commercially available benzaldehydes to form the C=C bond by the Horner Wadsworth Emmons Coupling reaction. The fluorinated analogue containing a hydroxyl substitute group was synthesised through the use of a para-methoxybenzyl protective group.



Successful synthesis of six trans-fluorinated stilbene analogues occurred utilising a two synthetic step procedure with varying yields. Compound characterisation and purification were carried out using TLC, ¹H-NMR, ¹³C-NMR, ¹⁹F-NMR and Mass Spectrometry. Production of the hydroxyl substitute fluorinated stilbene (SB6) was obtained impure with a low yield of 16%, through a deprotection method of refluxing with Hydrochloric acid and Ethanol. Therefore, modifications of this method or further investigations of more effective paramethoxybenzyl deprotection methods are required for future synthesis.

Four of the pure novel analogues (SB2-SB5) underwent initial *in vitro* anti-proliferation testing in four cancer cell lines within the Welsh School of Pharmacy, Tenovus Laboratories. Small growth inhibition was observed at a concentration of 100 μ M and was most significantly observed in the colon and prostate cancer cell lines. Further testing in a Wnt signalling reporter cell assay is currently being undertaken and these results could act as a drive in the development of a novel class of anti-cancer agents.

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What are the attributes of a good lecturer? The views of MPharm students at the Welsh School of Pharmacy)

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In 2009, the education report by Guardian scored the Welsh School of Pharmacy (WSP) as the highest of UK schools of pharmacy scored the highest 'average teaching score' with 89%.¹ In an international medical education guide, a number of roles and attributes of lecturers were identified including that of information provider.² WSP lecturers teach MPharm students from a variety of backgrounds. It is not known what lecturer attributes such students consider as being positive. The aim is to explore MPharm students' views on what may contribute to the attributes of a good lecturer in Welsh School of Pharmacy, Cardiff.

Ethical approval for this mixed method study was granted. The first stage was the use of unstructured open interviews with three lecturers and then based on these interviews and a literature search, semi-structured interviews with five MPharm students. All interviews were audio-recorded, with consent, and transcribed. Transcripts were read and coded to identify themes. Four main themes were identified from the interviews namely; the skills and knowledge of a good lecturer, out of class support, assessment and feedback and qualities of a lecturer: attitudes and behaviours. The questionnaire was piloted and a small number of minor changes were made. In the final questionnaire, there were 24 Likert statements with which students could indicate their level of agreement/ disagreement. Positive and negative statements were used. Questionnaires were distributed in lectures for Years 1-3. Questionnaires were emailed to Year 4 students. Quantitative data from the questionnaires were entered into a pre-prepared worksheet in the statistical package SPSS 16 for Windows XP. The Mann Whitney test was used to compare responses between groups. Results were considered statistically significant when $p=0.05$.

A 72.3% response rate was achieved (310/429). The three statements that most students indicated a level of agreement with (% agreeing together with % strongly agreeing) were those where 'A good lecturer...' '*...should provide accurate information.*' (98%), '*...should provide clear instructions and assessment criteria for work they set*' (98%) and '*...is one who is enthusiastic about their subject*' (97%). In addition, 'A good lecturer...' should '*...encourage students to ask questions.*' (86%) '*...inspire students to do as well as they can.*' (95% agreement) and '*...provide appropriate feedback in a timely manner.*' (95%). For other statements opinion was more divided that is 'A good lecturer...' '*...should finish the lecture late if they start late.*' (15%), '*...provide a lot of information 'one way' with no interaction from students.*' (15%) and '*...is one who lectures for 50 minutes without giving a break at all.*' (14%). There were some significant differences between responses from 1st and 4th year students, for example, the 4th year students indicated a significantly higher level of agreement with the statement "A good lecture provides a lot of information 'one way' with no interaction from students" ($U=2543$, $p=0.003$) than 1st year students.

Over 70% of the current undergraduate student population responded, which is an acceptable response rate. The 4th year student response rate was lower (56%) and this may have been due to the electronic distribution method and/or the 4th year being occupied with final year projects at the relevant time. The number of interviews was small but was useful in identifying topics for inclusion in the questionnaire. It would be useful for WSP staff to be provided with the results to make them aware of students' views on this topic. Suggestions for further work include the use of focus groups with students to explore reasons for student views, conducting surveys with other schools in Cardiff or other schools of pharmacy.

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Chemical and preliminary biological evaluation of poly[Ethyl Acrylate-co-Acrylic Acid]

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Wounds afflict all ages and sectors of society: from accidental abrasions to the intentional surgical incision, from sports related sprains and strains through to the ubiquitous ulcer, they are as variable as the patients that present them. A chronic wound is defined as 'a loss of tissue integrity which heals in an unpredictable timeframe'¹ and have the capacity to affect a large proportion of the population both now and in the future. Of greatest importance to this study was the role of bacteria in impeding the healing process. Several factors have been attributed to this inhibition: size of bacterial burden, bacterial virulence and the strength of resistance presented by the host². It has been found that bacteria including *Clostridii*, have the capacity to extrude Butyric acid, thus lowering the pH of the wound environment (as low as pH 5.4)³, further damaging the surrounding tissue and inhibiting the healing process. Poly[Ethyl Acrylate-co-Acrylic Acid] is a pH responsive polymer, it is also speculated to have antimicrobial properties⁴; at more acidic pH the co-polymer is in the active form, allowing it to reduce the bacterial burden so that an acidic pH cannot be maintained and the wound pH will tend towards physiological pH 7.4. The aims of this study were to authenticate previously synthesised polymer samples and evaluate the toxicity of the polymer to human fibroblast cells. It was not possible to analyse the antibacterial capacity of the polymer due to project time restraints.

Nine samples were provided from three subtypes, synthesised with three different monomeric ratios. These were authenticated by three techniques: ¹H-NMR spectra were generated to provide comparison with spectra from the point of synthesis to determine if degradation had occurred in storage, These spectra showed a contaminant which was probably generated during polymer synthesis. pH assessment was made to observe the extent of deviation from the optimum pH range of 7.5-7.8 for fibroblasts⁵. Solubility was assessed to assure that samples would remain in solution over the time periods necessary for latter cellular assays. It was decided that pursuit of contaminant identity and removal would benefit the study; Attached Proton Test and Distortionless Enhancement by Polarization Transfer-45 ¹³C-NMR were used to attempt to identify the impurity whilst the efficacy of a washing step to remove it was trialed with readily available laboratory solvents including ether and ethanol. Finally, polymer toxicity to fibroblast cells was considered by the affects of samples on proliferation and migration by application of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) dye reduction and Scratch assays respectively.

Authentication of polymer samples from ¹H-NMR spectra demonstrated that, in the time period between synthesis and study initiation, degradation had not occurred. However, a peak previously attributed to residual solvent⁶ was simply not plausible thus reassignment of this peak to 'contaminant' and subsequent investigation was necessary. ¹³C-NMR failed to identify the impurity. Treatment with ethanol wash and vacuum filtration successfully removed the contaminant. This was confirmed by ¹H-NMR. The low yield (26-33%) from this process and finite quantities of sample available meant that there would not now be enough clean polymer samples for cellular assay. The results of MTT and scratch assays were of limited value to the presence of contaminant though did illustrate potential optimum doses of two of the three sample subtype. Polymer pH evaluations suggested that the pH deviation in high concentrations may contribute to fibroblast toxicity, whilst the pH of purified polymer remained much closer to the optimum pH for fibroblasts: suggesting that firstly the contaminant may have been responsible for the alkalinity of samples and secondly the potential for greater fibroblast tolerance to clean polymer samples.

Conclusions of the study indicate great potential for the polymer. However, the limitations of the study include the presence of contaminant, time and the combination of limited sample available and low purification yield. The most valuable achievement of the study was the addition of the ethanol washing step to polymer synthesis, which will facilitate future polymer synthesis. Suggestions for future work include the synthesis and evaluation of a new pure polymer sample series to generate more reliably reproducible data.

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Development and validation of a health-related quality of life measure for adolescents with skin diseases

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There has been a great deal of research into the impact of skin diseases on patients' quality of life (QoL) both in adults and children. However, the research into the QoL of adolescents with skin diseases is still awaited. There is also a lack of QoL instruments specifically for adolescents with skin diseases¹ and thus there is a need to develop such an instrument reflecting the unique characteristics of this age group². The aim of this study was therefore to develop a health-related quality of life (HRQoL) instrument for adolescents with skin diseases.

Semi-structured interviews were conducted with a cohort of fifty adolescents recruited from the dermatology out-patient clinic of the UHW. They were asked to speak freely about the impact of their skin disease on their HRQoL and the interviews were then transcribed verbatim. The items were generated and developed into the first version of the questionnaire. The questionnaire was piloted to a cohort of twenty adolescents who also attended the dermatology out-patient clinic of the UHW. Together with their feedback and the feedback from four members of dermatology staff a revised version of the questionnaire was developed.

The data presented include part of previously published work with adolescents³. Fifty adolescents (M=17, F=33) with a mean age of 16 years (range =12-18 years) were interviewed. A total of 33 aspects of adolescents' HRQoL were identified which were categorised into 12 main domains with psychological domain being the most common (88%), followed by the impact of social life (74%) and leisure activities (74%). Some of the recurring individual aspect of HRQoL included fear of meeting with strangers (42%), effect on relationships and friendship (38%), and avoiding going out in public places (32%). Adverse effect on study and job opportunities was another important aspect for several participants (46%). From this a 32 item first version of the questionnaire was developed. Twenty adolescents (M = 8, F= 12) with a mean age of 16.5 years were asked to complete the first version of the questionnaire and to provide feedback. Five changes were made to the first version of the questionnaire due to the feedback from the patients and the four members of dermatology staff which included removal of 'feeling angry' (6), removal of 'feeling down' (4), changing 'isolated' to 'lonely' (4), changing 'burden' to 'difficult' (3) and changing 'pre-occupied' to 'think a lot' (3). This has now developed into a 30 item revised version of the questionnaire.

The results of this study have revealed that a number of aspects of HRQoL may be different or more important in this age group compared to those reported for adults and children. The issues expressed by patients showed the need for an adolescent HRQoL instrument, therefore the emerging themes from the interviews established proof of principle. This adolescent-specific HRQoL instrument will efficiently capture the impact of skin diseases in adolescents. This instrument can then be used in clinical trials as well as in routine clinical practice to aid the better management and treatment decisions of skin diseases in adolescents.

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Knowledge of substances by rugby union players in South Wales

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Rugby is the national sport of Wales with over 60,000 people taking part in 293 member clubs¹. Studies have shown that medical practitioners lack knowledge on the issue of drug misuse in sport² and doctors may be prescribing banned substances in sport inadvertently³. The Rugby Football Union has also expressed concern on the issue and has warned players that they are 'solely responsible for any prohibited substance found to be present in their body'⁴. It was decided to examine substance knowledge by rugby players in South Wales, and the use of pharmacists as sources of advice on banned substances in the game. The major aims were to identify the knowledge of banned and permitted substances among different standards of rugby union in South Wales and to establish whether the pharmacist has a role in educating players about prohibited substances.

Pilot interviews were chosen as an initial qualitative methodology for exploratory research into the issue. Questionnaires were then chosen to identify the level of knowledge possessed by players and any education/information received by players from their clubs. These questionnaires were administered personally to ensure completion with the use of gatekeepers for three professional/semi-professional teams. The data were entered into SPSS V.16.0 for Windows and presented using descriptive statistics. Secondary interviews were also conducted and designed to explore the sources of advice used for information on substances in the game, the use of pharmacists as a source of advice and additional services that could be provided to advertise information provision in pharmacies.

In total, 206 questionnaires were completed by players; professional/semi-professional (n=38), division 1-3 (n=102) and division 4-6 (n=66) players, from 11 clubs. The questionnaire response rate was 83.1% (206/248) which was increased by personal administration of the questionnaire along with supplying pens for completion. Responses showed that the knowledge of prescription drugs (47.1%) was lacking in comparison to drugs frequently misused in sport (91.4%) and OTC substances (78.6%). Professional and semi-professional players all obtained scores of 63% or above whereas distribution was more widespread for other standards. Knowledge of frequently misused drugs in sport was significantly higher (Mann-Whitney) in pro/semi-pro players than those in division 1-3 ($P=0.002$) and division 4-6 ($P<0.001$). There was also a significant difference (Chi-square) between pro/semi-pro players receiving information/education on banned substances from their club than those in div 1-3 ($P<0.001$) and div 4-6 ($P<0.001$). Pharmacists were the least likely person to approach for advice on substances in rugby (15.5%), whereas most were likely to consult their team doctor (52.9%). Nine interviewees were selected from different regions of South Wales that played at different standards of rugby union. Frequently misused drugs in sport were the main 'banned' substance identified by interviewees. Five of nine interviewees would not ask pharmacists for advice as they were 'unsure' if pharmacists had any, or sufficient, knowledge of banned substances in rugby union and some thought community pharmacists were inaccessible. There was a unanimous opinion that pharmacists should warn players when supplying products containing substances that are or may be subject to prohibition in sports.

This study was successful in identifying the lack of knowledge that South Wales rugby players have of prescription drugs and OTC products that are banned in the game. Improved knowledge of substances was shown with players that had received information/education provided by their clubs. Pharmacists are not used as a first source of advice on the issue of banned substances in rugby and simple changes such as offering services in pharmacies could change the perception of pharmacists and could lead to pharmacies being used more by rugby players seeking education in the future. Further studies could look at increased numbers of rugby players in a larger geographical area to investigate rugby player knowledge further and considering pharmacist views and knowledge of banned substances in rugby, along with their ideas of providing services to rugby players and other sports people.

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Design and synthesis of dual PPAR/RXR agonists for prostate cancer therapeutics

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Prostate cancer is second only to lung cancer as the leading cause of cancer related deaths in US men, where it is estimated that it causes up to 28,000 deaths each year. Although many patients can be treated with surgery or hormonal therapy, the disease can develop into hormone refractory prostate cancer which can be a very difficult condition to treat. The combination of docetaxel and prednisolone is the only chemotherapy regime licensed for use in hormone-refractory prostate cancer in the UK. This combination can cause substantial side effects and it may not be possible to use docetaxel if the disease has progressed to a stage where it is causing significant symptoms¹. As there is a high prevalence of the disease, few available treatment options, and problems with toxicity with current treatments, there is a clear need for the development of new chemotherapeutic strategies in this field. Peroxisome proliferator-activated receptors (PPAR) and retinoid X receptors (RXR) are members of the family of ligand regulated nuclear hormone receptors. Both of these receptors are recognised for having an anticancer effect based on *in vitro* studies². In order to exert its effects, the PPAR γ receptor subtype forms a heterodimer with an RXR α receptor subtype and then binds to specific recognition sites and regulates gene transcription³. The PPAR γ /RXR α heterodimer can synergistically signal several anticancer pathways, such as antiproliferation, induction of differentiation, induction of apoptosis and inhibition of angiogenesis making it a highly useful target for down-regulation of carcinogenesis⁴.

Known agonists of PPAR γ and RXR α can be used as a starting point for the design of novel lead compounds. Previous work by this group has identified substituted 2-benzylidene tetralones as potent inhibitors of CYP26, an enzyme which can metabolise retinoic acid (RA). Based on molecular modelling (MOE flexible alignment method), these inhibitors have similar structures to the PPAR γ agonist AZ 242 and the RXR α agonist 9-*cis* RA and may be used as a template for the design of potential agonists. Structure activity relationships (SAR) may result in optimisation of this lead compound.

In this work, the synthesis of a homologous series of compounds was attempted in order to initiate SAR studies. The synthesis included a range of medicinal chemistry strategies such as; 1) increasing the length of the lead compound to probe for optimum binding sites, 2) functional group interconversions and isosteric replacements in order to alter binding affinity and 3) comparison of chiral and achiral derivatives. The structures of the analogues synthesised were confirmed with ¹H-nuclear magnetic resonance (nmr), ¹³C-nmr, microanalysis and high resolution mass spectroscopy.

The aim of the project was to synthesise compounds for testing and to help explore and refine the methodology required for the successful synthesis of further analogues and intermediates. Some of the key synthetic methods have been established for the synthesis of 2-benzylidene tetralone analogues. A small library of derivatives were successfully synthesised and will be evaluated at a later date for biological activity. Molecular modelling (superimposition and MOE docking) suggest that these novel tetralone structures may be agonists of both PPAR γ and RXR α .

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The effect of Caveolin-1 on AKT/mTOR activity and Rapamycin sensitivity in prostate cancer cell lines DU145 and LNCaP

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Prostate cancer is the most frequently diagnosed cancer in men in Europe and the second most diagnosed cancer in men in the UK¹. The main treatment options are prostatectomy and/or hormone inhibition therapy. One of the major issues with prostate cancer progression is the cancer becoming hormone insensitive. Currently, there are very few curative treatment modalities for hormone insensitive prostate cancer². The activity of caveolin-1 and the AKT/mTOR signalling pathway may represent a significant target for the treatment of hormone-resistant prostate cancer. The mTOR pathway regulates cellular growth through the mediation of ribosome biogenesis and mTOR translation³. This pathway is made up of two parts; mTORC1 which is rapamycin sensitive and mTORC2 which is rapamycin insensitive. Currently there are a number of mTOR inhibitors, such as rapamycin, in clinical trials³. Caveolin-1 acts primarily through a caveolin-1 scaffolding domain as a negative regulator of kinases acting through recognition of caveolin-binding motifs present in signalling molecules such as EGF-R and ERK1/2⁴. In a study by Campbell et al.⁵ on renal cell carcinoma caveolin-1 binding motifs within RAPTOR, a component of mTORC1, and RICTOR, a component of mTORC2, were identified (unpublished). Here it was hypothesised the mTORC1 and mTORC2 are regulated by caveolin-1 with potential therefore of caveolin-1 modulating the actions of mTOR inhibitors like rapamycin.

Rapamycin sensitivity was analysed by treating cells seeded at 20,000 cm³ with varying concentrations of rapamycin (0, 1, 10, 100, 1000nM). DU145 cells were treated with caveolin-1 siRNA or luciferase siRNA. Cell growth assays or BCA tests were then performed to determine the effects of rapamycin on caveolin-1 down-regulation and the amount of protein required for Western blotting respectively. To assess the localisation of RAPTOR and RICTOR in relation to caveolin-1 a discontinuous sucrose gradient was used. Western blotting was used to probe cells for AKT/mTOR pathway markers caveolin-1, pS6, pAKT, pERK, pmTOR2448 and pmTOR2481 using specific antibodies to determine the effects of down-regulation of caveolin-1 had on this pathway and for the localisation assay. Caveolin-1 scaffolding domains were introduced into caveolin negative LNCaP prostate cancer cells with the aid of a cell-penetrating peptide (antennapedia). This was to establish what effect the introduction of caveolin-1 would have on prostate cancer cell growth.

DU145 cells treated with rapamycin showed a decrease in cell growth and LNCaP showed no general significant response to rapamycin. The down-regulation of caveolin-1 in DU145 did not show statistical difference in rapamycin sensitivity compared to the normal DU145 rapamycin treated cells. Western blots of siRNA treated DU145 cells showed caveolin-1 could be successfully down-regulated. This was accompanied by decreases in pS6 and pAKT. From the sucrose gradient it was found that caveolin-1 is not predominantly co-localised with RAPTOR or RICTOR. Introduction of caveolin-1 scaffolding domains via antennapedia displayed a trend of decreased cell growth compared to cell growth.

Caveolin-1 is present in rapamycin sensitive prostate cancer cells (DU145) but not in rapamycin insensitive prostate cancer cells (LNCaP). Caveolin-1 was found to positively affect the AKT/mTOR pathway as shown through targeted down-regulation of AKT/mTOR pathway markers pAKT and pS6. In caveolin positive DU145 prostate cancer cell line RAPTOR and RICTOR are not constitutively co-localised with the caveolin-1 enriched membrane domains in the absence of rapamycin. Although not statistically significant, it appears CSD reduces growth of androgen sensitive caveolin-1 negative LNCaP prostate cancer cells.

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Can the Theory of Planned Behaviour (TPB) be used to understand pharmacists' engagement with their Continuing Professional Development (CPD)?

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Continuing Professional Development (CPD) is a framework used by the Royal Pharmaceutical Society of Great Britain (RPSGB). It enables pharmacists' to enhance and develop their knowledge, skills and attitudes in their professional practice in the areas they identify as needing improvement¹. Once a pharmacist has completed their CPD they must then record what they have done using the RPSGB's online recording system. From March 2009 the RPSGB announced that every pharmacist registered in the UK must complete and record at least 9 CPD cycles annually. The Theory of Planned Behaviour (TPB) is a psychological theory that states the likelihood of a specific behaviour being carried out is directly linked to the individual's intention to perform that behaviour. The theory has already been successfully used to predict a wide variety of healthcare behaviours. The theory includes three major components: Behavioural Beliefs, Subjective Norm and an individuals' Perceived Behavioural Control. From these an individuals intention to perform a specific behaviour can be calculated. The study aim was to use the TPB to help understand pharmacists' engagement with their CPD². Objectives were 1) to investigate whether the components of the TPB can be used to describe pharmacists' attitudes and approaches to CPD, 2) to investigate all the barriers and facilitators to CPD and 3) develop a questionnaire based on the TPB to measure pharmacists' approach to CPD.

A semi-structured face-to-face interview schedule was developed based on the principles of the TPB. This allowed a more detailed exploration of issues raised by the respondent³ and allowed the researcher to probe into more detail about the attitudes, facilitators, influence of peers and helpful resources for CPD. Ethics Approval was gained before the study was conducted. Pharmacists were approached via email to gain interest in participating and following reply were sent an information sheet outlining the project. Due to the qualitative nature of the project it was essential that pharmacists displayed a range of different backgrounds and characteristics in order to represent the wider pharmacist population. The sample of pharmacists to be included were purposively sampled in order to achieve this and included in the study.

Eight pharmacists were interviewed from the South Wales area, six female and two male. These included pharmacist from the three main pharmacy sectors: Community, Hospital and Academia and in Community all pharmacists represented a separate employer. From the data analysis it became clear that pharmacists viewed the whole CPD process as two separate behaviours: 1) Undertaking CPD in general and 2) recording of CPD using the RPSGB's online system. Attitudes towards the two above behaviours were presented according to the three TPB components. 1) Most pharmacists' found that undertaking CPD was of benefit to their development as a pharmacist. Some reported that if their peers' outlook on CPD was more positive then this would alter their outlook. The main problems reported were; lack of time, struggling to identify learning needs and difficulty in engagement with CPD following a working day. Most pharmacists felt confident in their ability to perform CPD in general and a number of sources were used to help them identify learning needs. However, attitudes toward recording online were more negative. Common beliefs were that it was unnecessary and did not help with their ability to practice. However, they understood the need for recording their CPD. Pharmacists reported that many of their colleagues approached recording in a similar negative fashion. The main problem stated was that questions were difficult to answer and little was known about what the Society expects from them. Not enough information is provided on recording and this increases time required to complete the recording process. Being online has caused some pharmacists' problems as not all had access to the Internet at home.

This is the first study to use the TPB to explore pharmacists' professional development. Initial results based on eight interviews shows that the TPB is a useful model to explain pharmacists CPD behaviours. A detailed compilation of the barriers and obstacles pharmacists encounter while approaching CPD was elucidated as well as an in-depth insight into the beliefs that pharmacist had while approaching their CPD. The problems highlighted in this study could help the RPSGB to improve their online system and to ensure all pharmacists are able to fully engage in the CPD. From the data a draft questionnaire has been formulated with an inventory of statements that can be used to measure pharmacists' engagement with their CPD

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Reducing repeat medication errors in North Carolina nursing homes: What are the contributory factors and the association with subsequent patient harm

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In 2003, the State of North Carolina enacted a legislation requiring all licensed nursing homes in North Carolina to report medication errors to a reporting system. Since 2004, roughly 400 nursing homes subject to reporting requirements in North Carolina have reported nearly 70,000 medication errors to this web-based error reporting system¹. Medication errors are a major concern in North Carolina nursing homes, leading to an estimated 800,000 preventable medication related injuries every year in the U.S alone^{2, 3}. For every dollar spent on drugs in nursing homes, \$1.33 is spent on treatment of drug related morbidity and mortality. This amounts to \$7.6 billion for the nation as a whole⁴. In nursing homes, roughly 32% of residents take nine or more medications, compared with only 29% of community dwelling older adults that take 5 or more medications per day. Repeat medication errors are defined as the exact same error occurring multiple times to the same patient for the same reason. The aims of this study were firstly to determine the proportion of repeat errors, including number and type, occurring per nursing home, to assess the contributory factors relating to repeat errors, and to assess the association of repeat errors with subsequent patient harm.

This study was a non interventional, retrospective cohort study, where data used was collected using an online web-based system from September 2006 to October 2008. Data collected involved variables such as patient characteristics (age, gender, and cognitive ability), type of error, personnel involved, cause of error, and incident information. The individual medication error data collection form was sent to all 386 licensed nursing homes. A total of 25 out of the 386 North Carolina nursing homes were selected to pilot the new web system. The primary outcome in this study is repeat medication errors and the unit of analysis was medication errors, even though some nursing homes report more errors than others. The secondary outcome in this study was harm to patient. Severity is defined by 9 categories of error impact, ranging from near misses to death. Data was firstly analysed using descriptive analysis. Simple comparisons could then be made between new incidents and repeat incidents using a bivariate model. Secondly chi-square tests were carried out to produce multivariate model, enabling the author to calculate the odds ratios between individual variables.

The participation rate for this study was 76.2% (294/386). Out of the 15,037 reported errors, 5616 of these were repeat errors, accounting for 37.3% of all errors. Of the 294 nursing homes, the mean number of repeat incidents reported per home was 19.1. The contributory factors associated with the majority of repeat errors were those patients aged over 75 (67.8%), those patients cognitively impaired (70.2%), wrong dosage (65.1%), the 7AM-3PM shift (59.7%) and the documenting phase (50.8%). The multivariate model showed there was a 12.9% lower odds (OR= 0.871) of repeat errors occurring among cognitively unimpaired. The study also shows there was a 24.9% greater odds (OR= 1.249) that repeat errors were associated with harm.

Putting the 15,037 errors in context with respect to opportunities; for the 294 nursing homes in the State of North Carolina, the average nursing home receives 7.7 repeat errors per day. Several factors have contributed to causing repeat errors, but declined cognitive ability of patient's remains the primary factor, making this group the most vulnerable population due to their complex medication regimens and not having the ability to speak for themselves. This study has been proven successful in terms of identifying the high risk factors for repeat errors, and also in determining the significant impact of harm on patients suffering from repeat errors. Therefore success in the hospital setting should now guide similar efforts in nursing homes aimed at reducing repeat medication errors and improving the quality of care to this vulnerable population. For example, the benefit of the use of computerized technology in the hospital setting could be designed in long term care settings to focus on the documenting and administering errors in this study. Based on this experience in North Carolina, quality improvement efforts in nursing homes should now primarily focus on early identification of those patients with heightened risk factors (unable to self care, those older than 75 years), in order to prevent these errors from commonly repeating. This will subsequently lead to less harm to patients, hence improving overall patient safety.

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Risk assessment of point injection therapy as an alternative delivery method

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Point injection therapy is a method in which drugs, herbal extracts, vitamins and other fluids are administered into the body at an acupuncture point using a syringe and needle⁽¹⁾. It is considered to be one a complementary and alternative (CAM) therapy; and is practiced mostly by acupuncturists. Since the practice of CAM therapies is increasing, and there is no sophisticated system to regulate it, the risk of errors in practice can be great and may potentially lead to harm to patients. This project aims to apply NHS assessment of injectable products⁽²⁾ criteria to assess the performance of acupuncturists in the use of point injection therapy and then to develop a draft of a set of "Good Practice Guidelines" for the practice of point injection therapy for acupuncturists. This project additionally developed a method to determine the stability characteristics of a mixture of vitamin B1, vitamin B12 and Lidocaine; which is a commonly used combination of components used in point injection therapy.

An online questionnaire was distributed to registered acupuncture practitioners in the UK by email, the email addresses were gathered from the registration details on the British Acupuncture Council (BAcC) website. The questionnaire consisted of 5 sections and 27 questions, to gather details on basic information, preparation, administration, use of stability information, adverse effects, the discarding of the unused products and an opportunity for additional comment from participants on their practices. The questionnaire was designed to have multiple choice, yes/no answers questions as well as the possibility for free text entry in some questions. The questionnaire was put onto a website; an email containing the link was sent out using Gmail on the 1st March 2009, a reminder was sent on the 20th March 2009 and the online survey closed on the 1st April 2009. Responses were automatically transferred into report forms and sent to a defined email address. After the survey was closed, the report forms were processed manually and results were put into a spreadsheet for processing. In addition, a HPLC stability indicating assay⁽³⁾ was developed to analyse a mixture of vitamin B1, vitamin B12 and Lidocaine, this used a Thermo Spectra HPLC system with a C18 column and a UV6000 detector set for detection at wavelengths of 246nm and 360nm. Forced degradation tests were followed by stability testing over 24 hours period, with linearity and precision tests also run, whilst pH measurements were carried out during the 24 hours test.

From the 1662 emails sent, there were 276 responses (response rate 16.6%), in which 91 qualified reports obtained. It was shown that point injection was mostly practiced by Asian practitioners (75%) as part of private practice (76%). The most common (vitamin) preparation used was a combination of vitamin B1 and B12. The preparation was normally prepared in less ideal condition (non-aseptic), with nearly 60% of the practitioners not using stability information when combining drugs. 35% of practitioners do not label their products after preparation and 38.7% used unused doses for other patients, however, the majority of practitioners do make an effort to ensure that contamination is minimised during preparation, that all injectable products are used within 8 hours after preparation, whilst the practitioners tended to use cool place conditions (67.4%) rather than a fridge (18.6%) to store the preparations. In the HPLC stability indicating assay, it was found that the mixture was stable for 24 hours, however, this preparation is rather acidic (pH 2.79-2.83). The method showed good linearity and could be considered as precise (all %RSD less than 1%)⁽³⁾. In the assessment of injectable products⁽²⁾, a total of 5 / 10 high risks were identified in the practice of point injection amongst the acupuncture community.

Although the response rate is not high and the number of qualified responses is small compared to the number of acupuncturists in the UK, this is due to just a minority of acupuncturists practicing point injection therapy, whilst the number of emails sent, covered more than half of the registered contacts, therefore the outcomes are significant. The questionnaire was successful in identifying the risk factors which along with the lack of regulation in CAM therapy means a need for a focus on point injection therapy. As a result the draft "Good Practice Guidelines" was developed to help acupuncturists in the practice of point injection therapy. Additionally the HPLC experiment was successful in determining the stability of the mixture vitamins B1 & B12 with Lidocaine mixture; showing the method used to be convenient, precise and one that can be used as quantitative method for each ingredient.

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The pharmacology of b-phenylethylamine in the isolated ileum

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The recent identification of trace amine receptors proves that β -PEA have a neurotransmitter role in the GI system. It was thought that they are indirect acting sympathomimetic amines, but recent findings prove that they exert full effect on trace amine associated receptors. Previous thesis has discovered the pharmacology of TA in the guinea pig ileum. The aim of this study is to investigate the effects β -PEA in the presence of the antagonist (Mepyramine and Propranolol) including the desensitisation of the tissue with 5-HT.

Sections of Ileum were suspended in Krebs solution in the organ bath and electrical field stimulation (EFS) was applied. Cumulative concentration response curve were obtained for β -PEA repetitively and β -PEA in the presence of the antagonist as well as the response to 5-HT tissue desensitization all were recorded. The antagonist used was Mepyramine (10^{-6}) and Propranolol (10^{-6}) which was added in the organ bath and left to incubate with the tissue for 30 minutes. The desensitization of tissue with 5-HT (10^{-5} M), this dose was added repetitively three times to make sure the tissue has been desensitised, The Ratio of curves for control1(C_1) and control2 (C_2) was compared and analysis was done using paired students t-test.

There was a significant difference between the two curves. The antagonist and the 5-HT effects were analysed by taking the ratio of control 2 (C_2) divided by control 1 (C_1) and analysed with the ratio of curves of β -PEA in the presence of the antagonist and 5-HT tissue desensitisation, using ANOVA supported by Dunnetts post-hoc. β -PEA produced a contractile response in the EFS tissue, but there is a decrease in response compare with the first (control 1) to the second (control 2) , β -PEA contractile response, this is due to tissue desensitization with β -PEA and tachyphylaxis, as well as prolong contact of β -PEA in the tissue. In the presence of mepyramine and propranolol, there is a significant difference and the response to β -PEA was reduced but not abolished. β -PEA mediated a response after tissue desensitisation of 5-HT receptors.

The main finding from this study was, β -PEA does not mediate its effects via adrenergic, Histamine and 5-HT receptors. From the previous thesis, it was shown that Ketanserin abolished the response to β -PEA and might be mediating its effects through histamine receptors, but by the desensitisation of 5-HT receptors and using the antagonist to these receptors; β -PEA still mediates its effects.

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The effect of hypromellose capsule shell properties on the emptying of inhalation powders

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In the past few years, the research into the pulmonary delivery of active pharmaceutical ingredients (APIs) has been very active.¹ Dry powder inhalers (DPI) are commonly used to deliver drug to the lungs.² In DPIs, three types of container are used to hold the powder but generally, hard capsules are favoured for this application. There are two major types of hard capsule; gelatin and hypromellose. Several studies have been undertaken to compare the properties of gelatin and hypromellose capsules and these have proved the superiority of hypromellose capsules over gelatin capsules for the use in DPIs.³ This project focuses on hypromellose capsules, made using two types made from different grades of raw material, to improve their mechanical strength. The aim of this study was to examine the effect of change in the mechanical strength of the capsule shell on powder emptying.

Capsule samples were stored at 11, 33 and 56% relative humidities (RH) until equilibrium was reached to give a range of moisture contents. Samples were then punctured using a Foradil® DPI and the difference in the morphology of the puncture holes was compared using a binocular microscope. Further samples capsules, stored at 11% and 56% RH, were filled with about 20 mg of a powder formulation containing a known amount of salbutamol sulphate and using the DPI the weight of the drug released from the capsules was measured using the Andersen cascade impactor, followed by HPLC analysis.

Both types of capsule produced holes of approximately the same shape. At the higher moisture content, there was an increase in the number of irregular shaped holes in both capsule types. Holes became much more circular as the moisture content decreased. Powder emptying was measured on the samples with the lowest and highest moisture contents and no significant difference in the weight of recovered salbutamol sulphate was observed (one-way analysis of variance and Turkey's test, $p > 0.05$) between the two types of capsule. This means that the difference in the puncture hole morphology, which resulted from the difference in their moisture contents, had no influence on the powder emptying.

This study has shown that the change in the grades of raw material used to manufacture hypromellose capsules had no significant effect on the emptying of the powder from the capsules. This suggests that the mechanical strength of the capsule shells can be improved in order for them to withstand mechanical handling better on high-speed filling machines without affecting their powder emptying properties.

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Language choice in the pharmacy – What do future pharmacists think?

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Little is known about the impact of language barriers on the quality of care that patients with limited English proficiency receive in pharmacies.¹ Pharmacists are now operating at the frontline of the NHS and have an essential role in the treatment of illness. Their roles are expanding as never before and their ability to communicate with patients effectively is therefore essential. In line with the RPSGB's Code of Ethics, pharmacists are expected to provide advice and information for patients regarding their medications in a way that they can understand.² In the pharmacy setting, the impact of language barriers on medication use is particularly important, given the complexity of directions that patients receive, the serious implications of medication errors, the number of medications prescribed and patients' own responsibility for managing their medication.³ The aim of this study was to explore the individual opinions, attitudes and experiences of future pharmacists from the Welsh School of Pharmacy on the implementation of a language choice for patients in the pharmacy setting.

Since the issues that are of significance to this topic are not yet known, focus groups were chosen for investigative research into the attitudes of future pharmacists on the subject. Focus groups allow the researcher flexibility to probe participants and explore unanticipated themes and specifically use group interaction to generate data.⁴ Purposive sampling was used to recruit 21 undergraduate MPharm students. The participants were assigned into five groups of between three and seven on the basis of what language(s) they spoke and their level of pharmacy work experience. The participants were classed as a pre-existing group as they were already acquainted through working or socialising together.⁴ Focussing exercises were employed to stimulate discussion, including a news article with reference to health illiteracy and selected relevant points from the RPSGB Code of Ethics. All focus group discussions were audio-recorded, transcribed verbatim and subsequently themed and 'coded'. The method of de-contextualisation and re-contextualisation was then used to analyse the resulting data.

Many themes were identified. Several participants had experienced situations where a language problem had been a barrier to communication between a pharmacist and patient. These language barriers included problems with Welsh, Arabic, Chinese, Hindi, Punjabi and Urdu. The participants gave details of how they had overcome communication problems in the past, which included using family members as interpreters, talking slower, pointing, using diagrams, demonstrating with sign language, repetition and re-phrasing. Many of the participants expressed their concerns regarding these current methods of overcoming communication barriers. Numerous issues were raised with regards to using interpreters including confidentiality issues and interruption of the patient-pharmacist interaction; and with translating programmes, word-for-word translation bringing about different meanings to phrases. All of the participants showed an awareness of the seriousness of consequences for a patient experiencing a language barrier; with outcomes including side effects, hospitalisation, overdose and even death being raised. The issue of health illiteracy was also raised, in particular with regards to prescription labels and patient information leaflets. The majority of the participants believed that these services would be cost-effective if they were available in multiple languages, as many believed they would reduce hospitalisation rates for patients with language barriers. Many participants believed that patients would be able to better describe their symptoms, be more likely to comply with medications and be more likely to display question asking behaviour if they could communicate with the pharmacist in their preferred language.

Due to the exploratory nature of the study, the results were not intended to be generalised. However, this qualitative study using small numbers of future pharmacists uncovered a number of themes that highlighted key issues. The majority of participants acknowledged that the language needs of minority language patients should not be ignored and believed that offering a language choice for pharmacy services would increase the efficacy of healthcare and satisfaction for these patients. Now the main issues have been identified, further studies may include a questionnaire-style quantitative study using a 5-point Likert scale open to a broader sample of future pharmacists across all UK Schools of Pharmacy in order to confirm whether or not the participants' views in this study are representative.

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The synthesis of a novel anti-psoriasis agent using the co-drug concept; dithranol and quinazoline kinase inhibitor via a carbonate covalent linker

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Psoriasis is a chronic inflammatory autoimmune condition of the skin that affects approximately 3% of the UK population¹. Currently there is no cure, only symptomatic treatments. The medication available is of therapeutic value but this dermatological disorder characteristically presents in continuous periods of relapsing and remitting cycles of flare ups, making the need for new therapies. As the aetiology of the disorder is unknown¹, designing a novel therapy has proven difficult despite the target site of drug action appearing accessible; the skin. The aim of this project was to successfully synthesise a co-drug combining two potential anti-psoriatic agents.²

Overall, it has been proven that the use of a co-drug is a promising concept. It has been established that combining suitable compounds is challenging, but possible. The desired co-drug was not produced during this project, possibly due to stability issues but the results obtained will guide the further work that is required.

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A service evaluation of the information provision to mental health patients at Cardiff and Vale NHS Trust

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Mental illness affects one in four people at some point during their lifetime¹ and is becoming more prevalent. The obstacle of medication adherence remains a significant problem as relapse is a major concern¹. Providing education regarding medication to psychiatric patients potentially increases knowledge and adherence². However, medication information can be viewed as beneficial or detrimental dependent on the patient, which can be affected by factors such as age and diagnosis³. More medication information can empower some patients resulting in positive outcomes but can result in negative outcomes for others; inducing anxiety³. Patients use a variety of sources to answer their queries regarding therapy, therefore, it is imperative to determine how patients like to learn, how much and what information they require to ensure the service satisfies their needs. This project aims to evaluate the service of medication information provision to psychiatric in/outpatients of the Cardiff and Vale Trust.

Part I of the study involved a qualitative semi-structured interview with a staff member from the pharmacy department of the Trust. The interview aimed to find out about the current system of medication information provision. The results were used to inform the structured interview schedule used in part II of the study. Structured interviews were used to ensure high validity and good response rates.⁴ A purposive sample of mental health in/outpatients of the trust were interviewed face to face in a private room, where possible, to discuss the issues stated above related to medication information. Patients were asked to indicate their level of agreement to a number of statements on a 5-point likert scale as well as rank a variety of show cards according to criteria given by the interviewer. The quantitative data was input into Excel and presented in the form of frequencies and some statistical analysis, where appropriate.

Thirty patients were interviewed (n=30) and 57% (n=17) agreed they were completely satisfied with the way information was provided to them about their medicines, 27% (n=8) disagreed. Twenty five (83%) patients agreed that they had to ask for information about their medicines and most patients agreed that they would prefer information to be available without having to ask. Patients preferred sources of medication information were the doctor and the pharmacist due to their knowledge of medicines. However, only 8 (27%) patients had discussed their medicines with a pharmacist whilst at the trust. Twenty (66.7%) patients had received written information about their medicines with 35% (n=7) patients disagreeing that it was easy to read. Twenty-one (87.5%) patients would prefer to use more than one source to learn about their medicines.

This study, using a small sample, found that most patients were satisfied with the trust's information provision service. Reasons for dissatisfaction may be related to differences in patient information needs. Accessibility seems to be an area of dissatisfaction as the majority of people have to ask for information, which is not deemed appropriate by some. Few patients had discussed their medicines with the pharmacist, despite choosing them as a second most preferred source of information. Thus, it is imperative to improve patients' awareness of the pharmacists' role along with their accessibility. This study justifies a recommendation previously outlined by the pharmacy department of the Trust to have freely accessible medication information cards on wards and at clinics. Developing the cards in an easy to read format may also improve patients understanding of medicines information who struggle to read patient information leaflets. Studies into the impact of these medication cards on patient satisfaction would be an interesting area for further research.

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The use of scanning electron microscopy to investigate membrane ruffling caused by cell-penetrating peptides

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Macropinocytosis is one of the endocytic pathways that involve formation of cell membrane ruffling¹. Folding back of these ruffles forms macropinosomes that involve in the internalization of extracellular components. The use of cell penetrating peptides (CPPs) has attracted the interest of many scientists. Many studies have shown that CPPs has the ability to translocate across plasma membrane and has the potential of being efficient vectors in delivering small molecule drugs and macromolecular therapeutics². The CPP used in this study is known as R8, a short peptide consist of eight arginine residues. Microscopy has played an important role in many cellular studies but surprisingly the Scanning Electron Microscopy (SEM) is not widely used. Using SEM, the effects of R8 and its conjugation with the proapoptotic domain peptide PAD (klaklak)₂ were studied. Cell lines used in this study include HeLa, KG1a and A7r5.

In this study, cells were cultured and the required amounts of cells were added into 12-well plates containing glass covers in each well. HeLa and A7r5 adherent cells attach easily onto cover glass while KG1a suspension cells require poly-D-lysine treated cover glass to aid attachment. Cells were treated with designated CPPs at different concentrations for 5 minutes. Gluteraldehyde was used as a fixative. The cells were fixed overnight. On the next day a heavy metal fixative, osmium tetroxide, was used to further protect the cells. The cells were then dehydrated by passage through a graded ethanol series (50%, 70%, 80%, 90% and three times 100%, 10 minutes each). A critical point drying apparatus was used to replace ethanol with liquid CO₂ followed by a drying run process. The cells were then plated with gold in a sputter coater before being examined with a Philips XL20 SEM at 20-kV accelerating-voltage.

R8 peptides show increased formation of ruffles with increase concentration in HeLa and KG1a cells but not A7r5. Conjugation of R8 with PAD showed signs of abnormal structures in HeLa at incubation period as early as 2 minutes but cells remain healthy when treated with just PAD alone. Presence of serum in media used when cells were treated with peptides showed decreased amount of ruffles on plasma membrane of HeLa in contrast to those treated with serum free media. Incubation of peptides at different temperatures also showed differences in induction of ruffles. In HeLa, lesser amount of ruffles was observed when incubated at 4°C. At room temperature, ruffles were relatively more than those incubated at 4°C but lesser than those incubated at 37°C. Temperature has a lesser effect on KG1a cells. Relatively lesser ruffles formed in cells at 4°C but differences were not as prominent as HeLa. KG1a at 37°C and room temperature did not show a drastic variation. Formation of ruffles in A7r5 was surprisingly not much affected by temperature.

At higher concentration of R8, more ruffling was induced in HeLa. With the lesser effect of the peptide observed in KG1a and A7r5 might suggest that a specific peptide might have variable effects in different cell lines. Previous studies reported that actin reorganization was a function accompanied by macropinocytosis and cellular uptake of Tat-fusion protein was inhibited by treatment with amiloride, an inhibitor documented to inhibit macropinocytosis³⁻⁴. With the greater ruffling induced by treatment of R8 might suggest that the peptide was taken up by macropinocytosis as more actin reorganization forms more extension of cytoplasm. The structural changes shown in HeLa with the treatment of R8-PAD suggests cellular cytotoxicity as PAD was well known for inducing cell death by apoptosis. The effect of serum in membrane ruffling might be due to the capacity of the peptide to bind to serum proteins or degradation of peptides by serum enzymes, leading to lesser effective peptides available. Low temperature was reported to inhibit membrane ruffling hence experiments were performed. Results showed fewer ruffling at low temperature in HeLa. Despite the many cell studies using confocal microscopy, the use of SEM to study ruffling can be proposed to provide further evidence on the behaviour of surface membrane induced by CPPs.

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Comparison between two formulations of salbutamol; Airomir & Ventolin and their deposition in three different spacers; Aerochamber Plus, Nebuhaler and Volumatic

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Asthma and COPD treatment often involves regular inhaled preventer and/or reliever therapy. After the Montreal Protocol in 1987 which ordered that all inhalers phase out the use of CFC propellants, HFA propellants were discovered and new salbutamol formulations were developed¹. Salbutamol is an example of an inhaled β -agonist that is often used as reliever therapy for asthma and COPD. Two of the new salbutamol formulations are Airomir and Ventolin. Airomir contains two extra excipients, oleic acid and ethanol. Pressurised metered dose inhalers are often used to deliver these therapies although they do have some problems these include high deposition in the oropharyngeal region and some patients have difficulty in actuating and inhaling coordination². To overcome these problems spacers have been developed to aid the administration of salbutamol. Valved holding chambers are a type of spacer and will be used in this study, AeroChamber Plus, NebuHaler and Volumatic will be the spacers tested. The two different formulations of salbutamol will be compared along with the deposition in the three spacers. Modifications to the spacer and inhalation techniques will be investigated.

Three of each of the spacers (AeroChamber Plus, NebuHaler and Volumatic) were used to test two different formulations of salbutamol, Airomir and Ventolin. The amount of deposition in the three spacers were compared between each other and between the two formulations. The salbutamol was recovered from the spacer and quantified using HPLC and ChemStation software. After the two formulations were tested in the three spacers modifications to the spacers and inhaling techniques were tested to examine if any change in deposition was seen. AeroChamber Plus was tested with a slower inhalation and inhaling while actuating. NebuHaler was sealed in order that a partial vacuum was created in the spacer.

Ventolin had between 25-60% more of deposition compared to Airomir. AeroChamber Plus consistently had a higher amount of deposition than Volumatic and NebuHaler for both formulations. All methods showed some deposition with the method initiating inhalation before actuating of inhaler producing the lowest deposition with both inhalers. Slowing down inhalation only slightly reduced deposition for both inhalers, however creating a vacuum increased Ventolin deposition in NebuHaler.

The results indicate that the additional excipients in Airomir, oleic acid and ethanol do have an effect on the delivery of salbutamol via a spacer. All methods created deposition, with creating a vacuum being the least efficient therefore making it inappropriate to make this adjustment in future. Inhaling while actuating produced the least deposition making this a possible suggestion for the future, however deposition in the lung would have to be looked at when using this technique. The large volume spacers had lower amounts of deposition compared to the small volume spacer used in this study, indicating that the larger spacers would be clinically more efficient.

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An exploratory study into the role of the Welsh language in hospital pharmacy

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Communication is of vital importance in pharmacy practice¹. For effective communication to take place, a shared language must be spoken between the pharmacist and the patient². The 2001 census showed that 61% of people who live in the county Gwynedd, where the hospital involved in the study is located, speak Welsh as their first language³. Previous studies have shown that patients who speak Welsh as their first language feel more relaxed when speaking in their first language, and prefer to speak Welsh although they are capable of speaking English⁴. The aim of the study is to explore the views of hospital pharmacists and pharmacy technicians on the role of the Welsh language within hospital pharmacy. Study objectives include obtaining information on hospital policies concerning the Welsh language, and exploring the role of the Welsh language in the workplace, as well between pharmacy staff and patients.

The aim of the study was to explore the views of individuals, therefore a qualitative approach was adopted. The qualitative method chosen was a semi structured interview. An interview schedule was developed bearing in mind the aims of the study. Semi structured interviews were conducted with twelve pharmacy staff members purposively chosen with a variety of language backgrounds. A key informant interview was conducted prior to the interviewing process with the hospital's Welsh Affairs Officer. Information gained from the interview was used to inform the final part of the interview schedule on hospital policies/promotional schemes relating to the Welsh language. Other topics investigated were the use of the Welsh language in the workplace and the use of the Welsh language between pharmacy staff and patients. Interviews were audio recorded and transcribed 'ad verbatim' in preparation for thematic data analysis.

All study participants felt it was important to have Welsh speaking staff in the hospital. Welsh first language subjects felt that speaking Welsh gave them an advantage over non-Welsh speakers in that patients were willing to confide more with them. Although it is the hospital policy to establish the preferred language of patients, no formal method of establishing a patient's preferred language was reported. All subjects interviewed had experienced patients who either could not speak English, or had extreme difficulty in doing so. Study participants identified the elderly as the group of patients who are most likely to have trouble communicating in English. A general lack of awareness was observed amongst participants about hospital policies concerning the Welsh language. The majority of subjects interviewed were unaware that the hospital support staff who want to learn the Welsh language by arranging and funding courses for them to attend. One pharmacist who had learnt the Welsh language claimed that it has benefited her enormously, and that it gave her an advantage over non-Welsh speaking staff.

The results obtained from the study demonstrate a clear need for bilingual services in the hospital. Inability to offer Welsh services to Welsh speaking patients may result in vital information being withheld from the pharmacist. Pharmacy staff members should be encouraged to learn the Welsh language, if only a few words or sentences since it would benefit both staff and patients enormously. More Pharmacy staff members should be made aware that the hospital supports staff who wish to learn Welsh by arranging and funding courses for them to attend. Identifying and recording a patient's preferred language is pivotal if Welsh services are to be provided, and a formal, robust method of identifying patient's preferred language must be developed. Particular attention should be paid to the language needs of vulnerable groups identified such as the elderly. Pharmacy staff need to be made more aware of the hospital's policy concerning the Welsh language in order for effective operation of the policy to take place in the hospital.

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Caveolin-1 modulates the AKT/mTOR pathway and determines mTOR inhibitor sensitivity in endocrine dependent breast cancer cells

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Tamoxifen is currently first line treatment for patients with oestrogen receptor positive breast cancer¹. Around 50% of patients respond to the treatment. However, most of these patients develop resistance to tamoxifen which is a major clinical issue¹. It has been shown that AKT/mTOR activity is up regulated in certain types of breast cancer and so is considered to be a good target for anti-cancer therapies such as mTOR inhibitors (e.g. rapamycin)². Over-expression of AKT in breast cancers cells leads to increased resistance to tamoxifen but also to increased sensitivity to rapamycin and its analogues. As such mTOR inhibitors are proposed as a suitable treatment for tamoxifen resistant breast cancers³. Numerous studies have shown caveolin-1 to have both oncogenic and tumour suppressor roles in breast cancer and studies have linked caveolin-1 with the AKT/mTOR pathway. Therefore, targeting caveolin-1 may influence the activity of the mTOR pathway in breast cancer. This study aimed to determine i) the sensitivity to rapamycin, in the caveolin-1 positive and oestrogen-dependent WT-MCF7 and caveolin-1 negative and oestrogen-independent TAM-R breast cancer cells, ii) the effect of down-regulating caveolin-1 on the activity of mTOR signalling components in WT-MCF7 cells iii) the effect of down-regulating caveolin-1 on the rapamycin sensitivity of the WT-MCF7 cells.

WT-MCF-7 and TAM-R breast cancer cells were seeded at a density of 20,000/cm² in a 24 well plate for cell counting and a 6 well plate for western blotting. The cells were then treated with 0 nM, 1 nM, 10 nM, 100 nM, and 1 µM of rapamycin for growth assay and western blot analysis. WT-MCF-7 cells were treated with siRNA and were either probed for components in the mTOR pathway in western blot analysis or treated with rapamycin for a sensitivity study in which a growth assay was used to determine the effects. The caveolin-1 scaffolding peptide (CSD) was introduced to TAM-R cells by four different methods and a growth assay was used to determine which the best method was.

After treatment with rapamycin, WT-MCF-7 cells significantly decreased in growth, whereas TAM-R cells did not. Western blot analysis showed that WT-MCF-7 cells were caveolin-1 positive and TAM-R cells were caveolin-1 negative. siRNA treatment successfully down regulated caveolin-1 in WT-MCF-7 cells and as a result the activity of mTOR increased. The siRNA treated WT-MCF-7 cells also became insensitive to rapamycin, indicating that the presence of caveolin-1 correlates with rapamycin sensitivity. The four combinations used to introduce the CDS to TAM-R cells were compared in a growth assay and two combinations showed significant growth.

The results showed that i) WT-MCF-7 cells are sensitive to the rapamycin whilst TAM-R cells are resistant, ii) down-regulation of caveolin-1 in WT-MCF-7 cells increases AKT/mTOR signalling, iii) down-regulation of caveolin-1 in WT-MCF-7 cells results in increased resistance to rapamycin. Therefore caveolin-1 may be considered a key determinant of mTOR sensitivity in breast cancer. It can be concluded that mTOR inhibitors may not be useful in the treatment of tamoxifen resistant breast cancer and that caveolin-1 may be considered a key determinant and biomarker of mTOR inhibitor sensitivity in breast cancer.

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A study to determine whether NMDA receptors mediate astrocyte death via nitric oxide synthase activation in early ischaemia-reperfusion injury

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Ischaemia-reperfusion injury poses a serious problem with the treatments currently available for ischaemic strokes, which are all based on rapid reperfusion. Thus, drug treatments that could prevent the lethal signals that cause ischaemia-reperfusion injury are being investigated. Excessive glutamate release into the extracellular environment during ischaemic-reperfusion injury is thought to mediate both neurone and astrocyte death^{1,2}. Glutamate is thought to mediate this cell death via activating glutamate receptors, such as NMDA, in a process called excitotoxicity². This study investigates a possible mechanism through which glutamate may mediate cell death in astrocytes. We hypothesised that NMDA receptor activation mediates astrocyte death via nitric oxide synthase (NOS) activation in early ischaemia-reperfusion injury.

Firstly, MOG-G-UVW astrocytoma cells were subjected to simulated ischaemia-reperfusion (1 hour ischaemia: 1 hour reperfusion). Potentially protective drug treatments were administered either throughout the hypoxia-reoxygenation cycle or at reperfusion only. The drugs administered were; 10 μ M MK-801, an NMDA receptor antagonist; 1 μ M N-2-mercaptopropionyl glycine (MPG), a free radical scavenger; 100 μ M L-NAME, a non-selective NOS inhibitor; 10 μ M 3-bromo-7-nitroindazole and 100 μ M N²-propyl-L-arginine, both nNOS inhibitors; 100nM 1400W, an iNOS inhibitor; 0.024% Dimethyl sulfoxide, a solvent used. The purpose of this was to look at the effects of inhibiting NMDA receptors and NOS isoforms during simulated ischaemia-reperfusion. Also, the role of oxidative stress in ischaemia-reperfusion injury was observed via the administration of MPG, the free radical scavenger. Secondly, MOG-G-UVW astrocytoma cells were incubated with a range of glutamate concentrations at 37°C in an atmosphere of 5% CO₂ and 95% O₂, at standard humidity. This was to determine the effect of glutamate on astrocytes. MTS assays were used to determine cell viability in each experiment.

Glutamate was shown not to cause cell death in astrocytoma cells. Additionally, no statistical difference was found between the cell viability of the drug treatment groups and that of the hypoxia-reoxygenation control during simulated ischaemia-reperfusion.

Glutamate's inability to cause death in astrocytoma cells was unexpected as previous studies had shown that glutamate caused excitotoxicity in primary line astrocytes¹. This may be due to astrocytoma cells being more resistant to the effects of glutamate. Therefore, further experimentation on primary line astrocytes is required to determine if glutamate is toxic to astrocytes. MK-801 was shown not to be protective during ischaemia-reperfusion injury. This suggests that NMDA receptor activation is not involved in early ischaemia-reperfusion injury in astrocytes. Additionally, the NOS inhibitors and MPG were shown not to be protective in early ischaemia-reperfusion injury. This implies that the activation of NOS is not responsible for the astrocyte death observed. Also, the failure of MPG to attenuate astrocyte death shows that reactive oxygen species accumulation and oxidative stress are not a major mechanism of astrocyte death in early ischaemia-reperfusion injury. In conclusion, this study showed that NMDA activation does not mediate cell death via NOS activation in MOG-G-UVW astrocytoma cells in early ischaemia-reperfusion injury. Therefore, the hypothesis was rejected. However, the failure of glutamate to cause excitotoxicity in astrocytoma cells casts doubt on the value of the model as a true representation of an astrocyte. It is possible that changes in the astrocytoma cells may have made them more resistant to glutamate and resultantly the data obtained from this model would be misleading. It is possible that the hypothesis may still be true in primary astrocytes. Therefore, the data collected in this study alone cannot definitively show that the hypothesis would be untrue for astrocytes. There is clear need for further investigation into glutamate induced astrocyte death and the possible mechanisms through which it occurs in more appropriate models such as primary astrocyte cultures.

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Tyramine bronchoconstrictor response in the isolated trachea

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Tyramine is one of many biogenic amines, which are a group of naturally occurring amines that can exert significant effects as chemical messengers (i.e. hormones and neurotransmitters).^{4,1} The main pharmacological effect that the trace amines have is their ability to induce noradrenaline release from sympathetic nerve endings.⁴ Tyramine is present in cheese, certain red wines, aged meats and weight loss supplements.¹ Tyramine and other trace amines are synthesised from L-phenylalanine and L-tyrosine. The enzyme tyrosine decarboxylase is used. Tyramine is metabolised by mono-amine oxidase (MAO) enzyme specifically MAO-A.^{1,3} Previous investigations have shown that ingestion of large quantities of tyramine can lead to cardiovascular effects like an increase in blood pressure and heart rate.^{2,5} Tyramine can also relax and contract the airways.² Scientists have discovered the existence of a G-protein coupled receptor, (GPCR) known as trace amine-associated receptor (TAAR). Investigations have also suggested that TAAR receptor types may be responsible for the effects of tyramine and other trace amines.¹ The aim was to investigate the bronchoconstrictor effects of tyramine, to eliminate nitric oxide, β_2 -adrenoceptors and prostaglandins, which have relaxant effects that can mask contractions and to predict the respiratory effects of tyramine supplement use.

Tracheae were cut into a spiral and were isolated in the organ bath with krebs solution. The main aim was to record the response of tyramine before and after the addition of an antagonist. Concentration response curves (CRC) were constructed for histamine and tyramine in cumulative addition to the organ bath. Histamine was used as a control and to check whether the tissue was responsive. Responses were measured after adding each concentration. Responses were measured in grams tension and converted into percentage of the first tyramine response (before). Mean maximum (% first tyramine response) responses and dose ratios were calculated and compared using Student's t-test.

Tyramine produced two types of responses in the isolated trachea. The first type caused an initial small contraction at a concentration of 10^{-7} , followed by a relaxation at 10^{-4} . Further increase in the concentration lead to contraction. The second type of response caused a contraction at 10^{-7} and did not possess a relaxation characteristic. The mean ($n = 4$) % maximum response before the addition of the antagonist indomethacin (10^{-5}), a COX inhibitor, was 82.71 ± 9.99 %. The presence of indomethacin gave a mean % maximum response of 203.51 ± 115.21 %. L-NAME, a nitric oxide synthase inhibitor, produced a very small increase in the tyramine contraction at 10^{-6} to 10^{-4} . ICI 118,551, a β_2 -adrenoceptor antagonist did not have an effect on the tyramine responses. The antagonist cocktail, a combination of all three blockers, showed a small increase in the tyramine contraction at concentrations ranging 1×10^{-5} to 3×10^{-5} . The responses before and after the addition of the antagonist cocktail were significantly different ($P < 0.05$).

L-NAME had an insignificant effect on the responses to tyramine. It did not abolish the relaxation characteristic of the response. This implies that nitric oxide is not involved in the response to tyramine. The tyramine response in the presence of indomethacin shows a higher mean % maximum. This could be due to a result of blocking the more potent PGE (causes bronchodilatation),^{3,5} therefore allowing the bronchoconstrictor actions of PGF_{2a} to occur. β_2 -adrenoceptors can be eliminated from their involvement in the tyramine response, as ICI 118,551 did not have any effect on the response. The presence of the antagonist cocktail didn't affect the tyramine response a great deal. Seems likely that the contractile response to tyramine in the trachea is a direct action, possibly acting through the TAAR family of receptors. The results of this work and other investigations suggest that the quantities of tyramine in supplements may have the potential to cause bronchoconstriction in selected individuals.

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A potentiality for Authentic World for teaching pharmacology and pharmacy calculations to UGs and as a preparation for the pre-registration training year

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Mathematics is a key component in the pharmacy degree programme and an understanding of basic maths is essential.¹ Errors in medication dosage calculations (MDC) can lead to reduced clinical effectiveness or overdosing, which can be potentially fatal. Pharmacists are required to be 100% proficient with calculations and this is a skill that needs to be taught throughout the degree programme. The learning styles of students and the common areas of error need to be highlighted and rectified.² Authentic World (AW) is an innovative and interactive computer based software package that teaches calculations using a constructivist³ method. It is based on constructing mental images from information and previous experiences. The aim of this project was to obtain opinions from second year pharmacy undergraduates and Clinical and Pharmacology staff members regarding the use of AW at the Welsh School of Pharmacy (WSoP) and potentially during pre-registration

The questionnaires were designed with input from Dr. Sewell and the coordinator of AW, Dr. Simon Young from Glamorgan University. The students were provided with access to AW for two weeks prior to dispatching the questionnaires. The questionnaires were designed to find out staff and student views on maths and the use of AW at the school, and the presentation of the package. The majority of questions were closed (23 closed, 2 open). A 5-point centre weighted Likert scale was used to record the data. Pilot studies were conducted with student peers to check the validity, reliability and consistency of the questions. The staff members were informed of the study by a covering letter and a copy the questionnaire. Fifty nine out of 116 second year students and twelve members of staff completed the questionnaires. Ten final year students requested to participate and were given access to AW in addition to a questionnaire. ANOVA post hoc Dunnett's statistical tests were used to analyse the data generated.

The results showed that the participants agreed that there was a need for AW at the WSoP. Some staff members were finding it difficult to teach calculations and the majority of students (70%) without A-level maths found some difficulty in calculations. Overall, 64% of students felt that AW helped their arithmetic skills as well as increasing their confidence levels when performing calculations. Adaptations to the programme were needed such as more challenging questions and a greater variety of sections to cover all aspects of calculations in the degree programme. There was a general consensus from all cohorts that AW would benefit Pharmacology bench work by aiding dose calculations. Although many participants thought AW would be useful for pre-registration training, some believed it would not do so since pre-registration students are already exposed to the clinical environment.

The results indicated that AW would need to be introduced on a compulsory basis to allow students to gain maximum benefit from it. Students that had completed A/AS level maths may not have found medication dosage calculations difficult due to greater exposure to and practice of calculations. There was a general consensus that there is a need for AW at the WSoP, however, both staff and students wanted this to complement calculation workshops and the existing constructivist approach rather than to replace them. The majority of students and staff thought AW would benefit students of all four years. The students found the calculations in the package relatively easy and wanted them to increase in difficulty each year. Both staff and students felt strongly that the interactive features mimicked the clinical environment, but second year students may not have had sufficient clinical exposure to express a strong view. AW has proven to be of benefit to students by improving their ability to carry out calculations and improving test performances. Basic arithmetic needs to be introduced into the AW package to help students without A-level in maths. It can be concluded from this study that AW would benefit students at the WSoP if used in conjunction with workshop based and other currently employed teaching methods to enhance the active learning process.

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Evaluation of the use and efficacy of heparin nomograms in a university teaching hospital in North Carolina

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Anticoagulation with unfractionated heparin (UFH) is a high-risk therapy^{1,2}. Accurate dosing is difficult to achieve, especially due to inter-patient variability in anticoagulant response, high rate of medication errors with UFH and problems in monitoring heparin via activated partial thromboplastin time (aPTT) levels^{1,2}. To address these issues, weight-based nomograms have been developed which outline dosing and monitoring guidelines. Although these nomograms have marked an improvement, they have by no means eliminated the limitations associated with UFH therapy, and require review and development in order to optimise UFH therapy³. The study aims were to assess whether the current dosing nomogram at UNC Hospitals was being used accurately by healthcare staff, and whether the doses within the nomogram were achieving therapeutic anticoagulation. The study also examined the effects of age and gender on achievement of therapeutic anticoagulation, to determine whether these factors should be included as predictors of dosing within future nomograms.

The study was a prospective, non-interventional exploratory study carried out at UNC Hospitals over a period of six weeks. A convenience sample was used. Inpatients at UNC Hospitals, initiated on UFH therapy according to a nomogram, and receiving therapy for 48 hours or longer were recruited into the study. Patients were recruited by carrying out a daily data search using the UNC Hospitals' electronic pharmacy management program. This highlighted patients who had been initiated on UFH therapy within the previous 24 hours. Patients were screened according to inclusion/exclusion criteria, and eligible patients were followed up by collecting data from their heparin administration logs. Data was collected in an anonymous fashion on a standardised recording sheet.

A total of 59 patients (24 male and 35 female) were recruited into the study. Analysis of the data revealed a relatively high level of adherence, with 80% of aPTT monitoring and 89% of doses and adjustments performed correctly. However use of the nomogram was 100% accurate in only 8 out of the 59 patients. The mean time to achieve therapeutic anticoagulation was 21.5 hours, ranging from 6.3 to 87.5 hours. At measurement of first aPTT, only 36% of patients had therapeutic levels. By the second aPTT, 54% of patients had achieved therapeutic levels. Patients on the thrombosis nomogram took longer to achieve therapeutic aPTT levels. The mean therapeutic dose for the thrombosis nomogram found in the study (14.78 units/kg/hour) was considerably lower than the current nomogram dose of 18 units/kg/hour. Age was not found to have an impact in achieving therapeutic aPTT levels. Gender, however, did impact on anticoagulation, with females at higher risk of overdose and requiring lower initial doses, especially with the cardiology nomogram.

Accuracy of nomogram use is reasonably high, however mistakes are still regularly being made. With a high-risk drug like UFH, the aim should be 100% accuracy in nomogram use. Education of healthcare professionals involved in UFH therapy is key in improving accuracy of nomogram use. A major limitation of the study was the small study size, which combined with the high variability in response, failed to achieve statistically significant results. Therefore further research is required, especially regarding appropriateness of current thrombosis dosing. Consideration should be given to long-term outcomes to establish whether incorrect nomogram selection by physicians is a contributing factor to overdosing with the thrombosis nomogram. Gender was found to have an effect on the achievement of therapeutic anticoagulation and therefore further research should be carried out to more systematically assess the role of gender in achieving therapeutic anticoagulation and its possible inclusion in the nomogram as a predictor of dose.

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A longitudinal study to determine student attitudes towards pharmaceutical calculations

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A pharmacist's main concern is, and should always be, the patient's safety¹. Pharmacists and pre-registration pharmacy students will be relied upon to carry out pharmaceutical calculations during their training and even after they qualify. Patient safety is recognised as a priority for health care organisations¹. Pre-registration pharmacy students are required to demonstrate competence in pharmaceutical calculations by sitting a compulsory section in their pre-registration exam, which contains 20 questions and has to be passed at 70% without the use of a calculator². Students in general are reaching university with a lower level of numeracy compared to those students reaching university in the past³. The aim of this study is to investigate the perceived self-competence and confidence of fourth year pharmacy students at the Welsh School of Pharmacy in performing various calculations.

The research tool used for this study was questionnaires. There were three sets of questionnaires that were developed. Each questionnaire contained four sections. The first three being the same in each and the last section was different in all three. Questionnaires were handed out to students at three time points, in January 2008, in May 2008 following dedicated pharmaceutical calculation teaching and the last one in November 2008 during their pre-registration year. The first set of questionnaires was not analysed while the last two were analysed using SPSS version 16.

There were 104 students who returned the second set of questionnaires (May 2008) while 78 students returned the third set of questionnaires (November 2008) and 60 students returned all three sets of questionnaires. Results exhibited that students level of perceived self competence and confidence varied for different types of pharmaceutical calculations. It showed that between January 2008 and after the dedicated pharmaceutical teaching took place in May 2008 there was a statistically significant improvement in individual students numeracy skills while there was no statistically significant change in their numeracy skill between May 2008 and during their pre-registration year (November 2008).

Students should not be allowed to use calculators for performing pharmaceutical calculations at any stage during their MPharm degree then they will be competent and confident of performing these calculations when they sit their pre-registration exams which do not allow the use of calculators and during pharmacy practice as well. Estimation of answers is very important whether using a calculator or not, this is because it will give an idea of what the answer should be and if the answer is not something close to what was estimated then it might be wrong and it should be calculated again to get the right answer. Continued review and development of pharmaceutical calculation teaching within the MPharm degree which will aim to prepare future graduates for pharmacy practice.

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Molecular modelling and synthesis of potential nsP2 protease inhibitors of the chikungunya virus

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Chikungunya virus (CHIKV) is an enveloped, positive sense, single-stranded ((+) ss-RNA) virus. It is spread by mosquitoes of the *Aedes* species, particularly *A. albopictus* and *A. aegypti*, which have been known to cause widespread epidemics in Asia, Africa and the Indian Subcontinent for over 50 years¹. The World Health Organisation (WHO) estimates that over 1.2million people were infected in India and south Asia between February and October 2006 alone¹. In 2007, an outbreak in north-eastern Italy in which one death was reported², raised concerns about the likelihood of the virus spreading to other parts of Europe, including the United Kingdom. The illness is characterised by severe arthritis (which can persist for weeks to years), rash, fever and headache. There is currently no antiviral treatment or vaccine available for the Chikungunya virus. The viral genome codes for structural and non-structural proteins. The non-structural proteins (nsP1-4) play a vital role in viral RNA synthesis, with the most important being the nsP2 which contains the Cysteine protease that cleaves the large polyprotein precursor to give the individual non-structural proteins³. The aim of this research was to study the interactions of potential inhibitors of the nsP2 cysteine protease of CHIKV with the active site, synthesise them and evaluate their ability to inhibit viral replication and as such prevent transmission of the virus.

Using molecular modelling software (MOE)⁴, the 3D homology model of the CHIKV nsP2 protease was developed and a database of protease inhibitors was screened against the active site of the CHIKV protease to determine the desired properties of a lead compound. A small library of compounds was designed based on alterations to the lead compound. The compounds were docked into the binding pocket using FlexX and potential interactions were viewed in MOE. Synthesis involved a classic Passerini 3-component reaction of a carboxylic acid, a carbonyl compound and an isocyanide in a one-pot procedure.

Six of the docked compounds had good orientation within the tight binding pocket and showed good ligand interactions. These compounds were successfully synthesised as three methyl ester analogues and three amide analogues, purified by flash column chromatography and characterized by Nuclear Magnetic Resonance (NMR) spectroscopy.

Although the synthesis was a success, the reactions were time-consuming and achieved low yields. Further investigation into ways to optimise reaction conditions to reduce reaction time and increase efficiency is needed. With increase in global warming, globalisation of trade and migrant travel, the geographic range of the culprit mosquitoes is on the increase and it is likely that more epidemics are inevitable which pose a worldwide public health problem. In addition to control and prevention measures which are currently being implemented in different parts of the world, public health education is crucial.

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An analysis of prescribing rates of Buscopan® and OTC sales rates of Buscopan® IBS Relief 2003-2008

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Over-the-counter (OTC) sales of medicines have been estimated to be equivalent to one third of the NHS drugs bill¹. Buscopan® IBS Relief was reclassified from POM to P in 1991, and from P to GSL in 2005. Studies show that people in more deprived areas are less likely to purchase medicines OTC however, it has been proven that cost is not the only influence in purchasing OTC medicines². Misuse of OTC medicines exists, generally in more deprived areas, and Buscopan® IBS Relief has not previously been associated with misuse³. Increasing availability of medicines may result in changes in patterns of prescribing and sales. The aims of this study were to determine the impact of the abolition of the prescription charge in Wales and whether use was influenced by deprivation.

A cross-sectional comparative analysis of OTC data for Buscopan® IBS Relief for all Welsh Local Health Boards (LHBs) and selected English Primary Care Trusts using data from October 2003-September 2008 was undertaken. Dispensing data for October 2003-September 2008 was used for the analysis of prescribed Buscopan® for Wales only. Data was standardised per 1000 population. The Welsh Index of Multiple Deprivation (2005) was used to rank each LHB in order of deprivation. Non-parametric descriptive statistics were used.

The rate of sales and the rate of prescribing increased for each study group over the period studied. When considering the abolition of the prescription charge in Wales, Wilcoxon Signed rank identified statistically significant difference between September 2005-April 07 and May 07-September 2008 for both the rate of sales ($p < 0.01$) and rate of prescribing of Buscopan® ($p < 0.01$). A significant difference was found between the most and five least deprived LHBs and the rate of prescribing of Buscopan® ($p = 0.01$) and the rate of OTC sales of Buscopan® IBS Relief ($p = 0.02$), identified using Mann Whitney tests.

The increase in the rate of sales of Buscopan® IBS Relief may be related to its reclassification to GSL status in 2005. The variations in the rate of sales of Buscopan® IBS Relief and the rate of prescribing of Buscopan® between more deprived and less deprived areas may be caused by changes in health seeking behaviour or use of Buscopan as an adjunct in opiate withdrawal. As both prescriptions and sales rates rise significantly post abolition of the prescription charge, it is difficult to conclude the effect on switching from sales to prescription.

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Activation of ErbB3 (HER3) receptor in experimental and clinical breast cancer

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The ErbB receptor family members ErbB2 and EGFR have been shown in the Tenovus Centre to be induced by antihormones¹ e.g. fulvestrant in oestrogen receptor (ER) positive MCF-7 breast cancer cells. This provides compensatory signalling that ultimately allows emergence of resistance. Such receptors commonly undergo heterodimerisation with further ErbB family members to allow activation of downstream signalling kinases,² but it remains unknown if ErbB3 is also antihormone-induced and contributory to compensatory signalling in breast cancer cells. If proven, this could explain recent observations that a key ErbB3 ligand Heregulin has considerable capacity to overcome antihormone response. This project aimed to provide new information on level and localisation of ErbB3 activation in breast cancer cells during antihormone treatment in vitro and to monitor ErbB3 activation within clinical breast cancers.

Firstly Western Blotting was used to investigate the level of expression and activation of ErbB3 (Ty1289ErbB3 antibody) and the downstream signalling kinases AKT and ERK in MCF7 cells treated +/- fulvestrant (10^{-7} M) and with Heregulin (0-10 μ g). Fulvestrant enhanced heregulin-activated ErbB3 and its downstream cell survival kinase AKT. Secondly two immunocytochemistry assays were developed and optimised using the Tyr1289ErbB3 antibody for cell line and clinical breast cancer studies. Immunocytochemistry for cell lines was optimised using MCF7 cell line +/- Heregulin. Following optimisation the assay was then applied to the cell models, MCF7, T47D (ERpositive/ErbB2 negative) and to BT474, MDA361 (ERpositive/ErbB2positive) with and without 7day fulvestrant treatment (10^{-7} M). A range of cell lines were used to better reflect the status and range of cancers in the clinic.³ An immunocytochemistry assay for clinical breast samples was then applied to a series of 14 paraffin embedded clinical samples donated from Nottingham City Hospital. Parallel clinical data were available for activated ErbB2, MIB1(a proliferation marker) and ER status.

Using a range of ER+ve models reflecting key breast cancer clinical sub-types (MCF-7, T47D: ErbB2-ve; BT474, MDA361: ErbB2+ve), the cell line assay determined that heregulin activated ErbB3 at the plasma membrane, while fulvestrant induced a significant increase in activation of ErbB3 in the nucleus ($p=0.005$) and the cytoplasm ($p=0.003$) in ErbB2-ve models and at the plasma membrane ($p=0.003$) and cytoplasm ($p=0.005$) in the BT474 ErbB2+ve model only. The optimised activated ErbB3 clinical assay applied to 14 breast cancers stained 65% of samples, with localisation at the membrane, cytoplasm and the nucleus and staining heterogeneity between samples. It was also found in the paraffin embedded clinical samples that activated ErbB3 was co-expressed with activated ErbB2 receptor ($p=0.005$) suggesting heterodimerisation.

The findings indicate that ErbB3 is commonly induced by antihormone in both ER+ ErbB2- and ER+ ErbB2+ cells and that this enhances signalling of Heregulin, an event which may drive resistance where this growth factor is available in the paracrine environment. This implies that new therapeutic approaches under development that target ErbB3 as well as ErbB2 and EGFR (e.g. pan-erbB inhibitors) should be examined alongside antihormones to see if they improve growth inhibition. The successful development of immunocytochemical activated ErbB3 assays should also allow this concept to be monitored in clinical disease in the future.

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Determining the minimal important difference of the Dermatology Life Quality Index (DLQI) and the Family Dermatology Life Quality Index (FDLQI)

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Health-Related Quality of Life (HRQoL) instruments such as the DLQI and FDLQI are useful in assessing how different skin conditions affect patients' and family members' lives, to assess the efficacy of patients' treatment over time and as an additional measure at assessing the risk-benefit ratio for certain drugs. If however any decisions are to be made on the basis of a change in HRQoL score over time, then there needs to be a definition of what change in score is meaningful to the patient, or family member in the case of the FDLQI. The smallest change in score over time that would be meaningful to the patient (or family member) is called the Minimal Important Difference, or MID. The aim of this study was to determine the MID for the DLQI and the FDLQI.

Patients who had recently started or were about to start a new treatment were recruited from the dermatology department in the University Hospital of Wales in Cardiff. Patients and family members were requested to fill in the DLQI and FDLQI respectively, along with disease specific questionnaires for psoriasis: Impact of Psoriasis Questionnaire (IPSO) for patients and Psoriasis Family Index (PFI) for family members, eczema: Quality of Life In Atopic Dermatitis (QoLIAD) for patients and Dermatitis Family Impact questionnaire (DFI) for family members, and acne: Acne-Specific Quality of Life questionnaire (Acne-QoL) for patients. A physician's assessment of the disease severity was performed along with disease specific severity scoring techniques for psoriasis: Psoriasis Area and Severity Index (PASI), eczema: SCORing Atopic Dermatitis (SCORAD), and acne: Leeds Acne Grading Technique. Correlations between these disease specific instruments and DLQI and FDLQI scores were analysed using a Spearman's rho test. A combination of anchor-based and distribution-based approaches was used. The anchor based method chosen was the use of a Global Rating of Change questionnaire; one clinical and one quality of life. The change in DLQI and FDLQI scores over time was then linked to these external anchors to determine the MID.^{1,2} The distribution based approach chosen was the use of effect size and standardised response mean calculations, these both calculated the MID using statistical characteristics of the sample.²

A sample of 79 patients and 33 family members completed the first stage of the study, with 36 patient and 10 family member follow ups. The mean age for patients was 35.32 years (SD 18.186), and the mean age for family members was 42.68 years (SD 10.997). A good cross section of skin diseases was studied (19 different skin conditions) ranging from benign lesions to extensive adverse drug reaction rashes. Correlations were found between the DLQI and IPSO ($r_s=0.8$, $p=0.000$), as well as the DLQI and Acne-QoL ($r_s=0.81$, $p=0.000$), and the DLQI and patient's global assessment of disease severity ($r_s=0.7$, $p=0.000$). The statistically significant differences in score from baseline to second administration of the DLQI ($p=0.000$) and FDLQI ($p=0.001$) were found. The MID of the DLQI was found to be between 0.7 and 3.2, and the MID of the FDLQI was found to be between 0.52 and 3.5

The predicted MID for the DLQI fits in with past attempts at defining the MID for the DLQI in specific conditions such as urticaria³ and psoriasis⁴. These predicted ranges were found to be quite broad, but this may have been due to the fact that such a broad range of skin conditions of varying severity and duration was studied. Skin lesion patients were found to have a generally lower score in the DLQI as did their family members on the FDLQI, whereas patients suffering from conditions such as psoriasis and adverse drug reactions and their family members were found to have higher DLQI and FDLQI scores. Since patients' quality of life is often related to their family members' quality of life it is important to measure the impact of a patient's skin condition on their family. The predicted range for the MID of the DLQI and FDLQI may be applied in practice and in research and will hopefully aid in clinical decision making. As there is now a definition that applies to more than one skin condition the hope is that the DLQI and FDLQI will be used more in practice and in research as an additional measure of treatment success or failure.

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An exploratory study to investigate learning and teaching of medicines-based calculations

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Medicines-based calculations play a fundamental role in the administration of medication throughout various healthcare professions¹. The teaching and learning of these calculations throughout university is therefore vital as the development of these skills will play a significant role in forming a competent practitioner. It has been claimed that when calculators are prohibited in classes where calculations are taught, students levels of confidence decrease and they experience difficulty when performing simple arithmetic². The aims of this study were to investigate the teaching and learning of medicines-based calculations at the undergraduate level, as well as investigating the level of confidence and self-perceived competence of students when dealing with numerical operations including medicines-based calculations.

Focus groups were chosen as a qualitative method of research into the opinions and perceptions of students regarding their experiences during the learning and teaching of medicines-based calculations. First year students from the MPharm and Nursing degree courses were recruited and specific arrangements were made for groups of students to attend focus groups at times suitable to them. A semi-structured interview schedule was developed and used throughout the focus groups to stimulate discussion amongst the participants. The semi-structured interview schedule consisted of two major sections: i) mathematics experience prior to university, ii) mathematics experience following commencement of the respective course. A pilot focus group was carried out to check the validity and reliability of the interview schedule. In total, six focus groups were implemented, with five groups for pharmacy students and one for the nursing students. Each focus group was recorded and transcribed, with the transcripts being read and re-read to find themes and sub-themes within the data.

In total, 29 students volunteered to take part in the study. 22 (76%) of the participants were pharmacy students and 7 (24%) were nursing students. The main themes to emerge throughout the focus groups were that students felt comfortable in their ability to handle basic numeracy, and that calculators are integral to the students level of confidence. Many students revealed the limited experience they had without the use of a calculator and stated that they would find it difficult to perform numerical operations without a calculator. Students also claimed that a constructive environment such as the workshop is the best method of teaching medicines-based calculations. Students who had taken mathematics for A-level seemed to have a greater level of confidence when approaching calculations, in comparison to those students who had only taken mathematics to GCSE level.

Students may find it beneficial if more time was dedicated to teaching medicines-based calculations in a constructive environment. Where medicines-based calculations are the main area of interest in a workshop, calculators should not be permitted in order to increase the levels of confidence of students. Providing students with further opportunities to practise calculations during their degree may increase the levels of competence when performing them in practise. Future studies could target the types of assessment used to evaluate a student's numerical competency as well as investigating throughout all four years of the MPharm degree.

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Patients' perspective of the Medicines Use Review (MUR) service in Wales

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A Medicines Use Review (MUR) is an advanced level service of the contractual framework, introduced in April 2005 to community pharmacies in England and Wales¹. A MUR is a concordance based review with the primary aim of determining patients' use of medication, both prescribed and purchased². MURs aim to include the patient in the discussion and decision making process regarding their medicines². The aim of this study was to establish patients' perspectives of the MUR service.

Qualitative one to one interviews were carried out with ten patients, identified by community pharmacists working in four Lloydspharmacy[®] community pharmacies in the Torfaen Local Health Board (LHB) area. Employing a qualitative study design enabled a range of opinions, experiences and suggestions to be identified from patients³. Patients were invited by the respective community pharmacists to take part in the study after having a MUR between 16th February and 2nd March 2009. Interviews were audio recorded and transcribed *ad verbatim* for thematic analysis.

Six main themes and several sub-themes were identified. These were 1) Role of the pharmacist 2) Information about medicines 3) MUR 4) Consultation area 5) After the MUR 6) Suggestions for improvement. Many of the themes identified mirrored findings from other studies concerning MURs^{4, 5, 6}. Patients showed signs of confusion regarding the name "Medicines Use Review", with several reporting that resolving these issues would help improve the service. Patients claimed to have gained knowledge about medication as a result of a MUR and generally had a positive experience. Patients appeared to understand the purpose of a MUR. Suggestions for improvement primarily involved renaming the service and differentiating a MUR with a pharmacist from a medication review with a GP. The themes identified will be used in future as a basis for the development of a structured questionnaire. Development of a questionnaire is essential to gain further insight into patient satisfaction with the MUR service. Future research should include samples from large multiple community pharmacy chains and independent community pharmacies. Where possible several community pharmacies from each LHB area in Wales should be included, this will allow generalisation of results.

The findings from this research should be considered when reviewing the service in order to maximise the benefits and experience of those making use of the service, thus encouraging others to partake. Establishing factors that are important to patients regarding the MUR service can aid development of additional advanced services for community pharmacy.

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The impact of ophthalmic chloramphenicol reclassification on prescriptions for chloramphenicol eye drops and ointment

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In June 2005, chloramphenicol eye drops 0.5%¹ was reclassified from POM to P. This was followed by the reclassification of chloramphenicol ointment 1% in September 2007. The basis for the reclassification, in addition to meeting the criteria of the Medicines and Healthcare products Regulatory Agency (MHRA), would allow patients quicker access to treatment for acute bacterial conjunctivitis³. Other studies have examined the impact of a reclassified medicine on the number of prescriptions for the same drug on prescription⁴ but no such studies have been undertaken for ophthalmic chloramphenicol. As a consequence it was of interest to study the impact of the reclassification of ophthalmic chloramphenicol in Wales on the number of prescriptions generated in primary care and also determine the impact on the sale of propamide and dibromopropamide ophthalmic preparations, the only preparations available for sale for the treatment of eye infections prior to the reclassification of chloramphenicol.

A retrospective analysis of Welsh primary care prescription data for ophthalmic chloramphenicol and OTC sales data for ophthalmic chloramphenicol, propamide and dibromopropamide was undertaken. Prescription data were collected from April 2004 to August 2008 and OTC sales data were collected from September 2005 to August 2008. Data were plotted at monthly intervals, using descriptive statistics (median and interquartile range), standardised as items per 1000 population, and differences explored using the Wilcoxon Signed-Ranked Test. Graphical presentation and statistical analysis were generated with Microsoft Excel 2003 and SPSS version 16.

Prescriptions for ophthalmic chloramphenicol showed a seasonal variation with a peak between December and April each year. This trend was not observed with the sales of OTC ophthalmic chloramphenicol, propamide or dibromopropamide. The change in the number of prescriptions for ophthalmic chloramphenicol in the 12 month period before (year 1) and the 12 month period after (year 2) the introduction of OTC ophthalmic chloramphenicol was not significant (3.66 [3.09-4.37] vs. (3.54 [3.06-4.37], $p=0.182$). There were also no difference in the number of prescriptions between year 1 and 3 (3.66 [3.09-4.37] vs. 3.54 [3.02-4.31], $p=0.182$) and between year 1 and 4 (3.66 [3.09-4.37] vs. 3.59 [2.84-4.23], $p=0.182$).

The number of prescriptions for ophthalmic chloramphenicol was not reduced by the reclassification of ophthalmic chloramphenicol from POM to P. In contrast, the sales of OTC ophthalmic chloramphenicol indicated an overall increase in the usage of ophthalmic chloramphenicol and had little effect on the sales of OTC ophthalmic propamide or dibromopropamide. This suggests the reclassification of chloramphenicol created a new market for the OTC preparation. The present study revealed little about the individuals who purchased chloramphenicol and this is the clear disadvantage of undertaking a quantitative study. It would therefore be useful to follow this study up with a qualitative piece of work.

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Quantitative structure activity relationship (QSAR) for maximal transdermal flux from experimental data

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The maximal flux (J_{max}) of a compound across skin from saturated vehicle is of interest from the viewpoints of transdermal delivery and toxicology. Magnusson et al¹ concluded that molecular weight (MW) is the main determinant of J_{max} . However the authors often estimated J_{max} as the product of permeability coefficient (k_p) and solubility. The present study uses only experimental J_{max} values to test the validity of their conclusion. A dataset of 115 compounds was extracted from the literature using strict inclusion criteria. Regression analyses showed that (1) MW an unreliable predictor (2) no combination of simple predictors (P - octanol/water partition coefficient; HAcc, HDon - Hbonding acceptor and donor numbers respectively; S - molar aqueous solubility) was effective. The dataset was then split into three groups: High J_{max} : group 2 ($\text{Log } J_{max} (\text{mol}\cdot\text{cm}^{-2}\cdot\text{hr}^{-1}) = > -7$), Medium J_{max} : group 1 ($-9 < \text{Log } J_{max} < -7$) and Low J_{max} : group 0 ($\text{Log } J_{max} = < -9$). Cross-validated multivariate discriminant analyses suggested that high J_{max} was associated with: MW = 160, HAcc = 3, LogP 02, Log S = -1.7. Discriminant analysis showed more potential in prediction of for transdermal flux.

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Targeted delivery to Langerhans Cells in human skin via microneedles

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Microneedles have been proposed as useful alternative to facilitate cutaneous gene delivery to humans. Gene delivery through traditional transdermal formulation has been proven difficult due to the extremely low permeability of the skin.¹ Microneedles are capable of breaching the external barrier imposed by the skin, hence allowing delivery of charged molecules and DNA.² The arrays of the microneedles (MN) are built to permeate the skin barrier to create microconduits without stimulating the pain receptors in the dermis.³ Thus, they are able to circumvent the delivery barrier in a non-invasive manner.⁴ The aim of the project is to investigate the potential of delivering DNA via MNs to the skin for cutaneous gene therapy applications. The project also determines whether DNA delivery via MNs can result in an immune response.

The following methods were performed in order to achieve these aims. (i) Transepidermal Water Loss (TEWL) study to determine the TEWL variability and reproducibility. The experiment also helps to establish the correlation between TEWL and skin barrier function. (ii) Extracellular Delivery of Hepatitis B Surface Antigen (HBsAg) to human skin via microneedles. (iii) Intracellular delivery of pRc/CMV-HBs(S) (Plasmid DNA) to the skin via MNs. (iv) Optimisation of MNs Coating Technique. (v) Investigating the role of TNF- α in Immune Response System. (vi) Investigating the response of Langerhans Cells to H5 Antigen.

The TEWL study has established a correlation between elevations in relative TEWL and the degree of skin barrier disruption through successive MNs administration. The extracellular delivery of HBsAg to the skin via MNs was unsuccessful. The experiment was not repeated due to time constraints. The intracellular delivery of pRc/CMV-HBs(S) (Plasmid DNA) has successfully resulted in HBsAg expression in the skin samples. A channel dip coater was made and satisfactory results have been observed in the experiment conducted to optimise MNs coating. Apart from that, the delivery of pRc/CMV-HBs(S) and HBsAg has failed to show an upregulation of TNF- α . The H5 antigens delivery to the skin via microneedles caused Langerhans cells response.

The results from TEWL study have implied that the MNs treatment should ideally be measured on one particular area where the measurements are reliable. Additionally, the TEWL study also indicates that MNs administration can effectively disrupt the skin barrier and form micro-channels suitable for drug administration. This would serve as a fundamental platform for the use of microneedles to deliver macromolecules (proteins, DNA) into the skin. The HBsAg expression has implied that the gene has been incorporated into the human cells when delivered using microneedles. Thus, this indicates that gene delivery to human skin via MNs is feasible. The results from the MNs coating were significant in allowing optimal delivery of antigens to the skin. Despite the absence of TNF- α upregulation following the delivery of pRc/CMV-HBs(S) and HBsAg, suggestions have been made to improve the delivery of the vaccines. Langerhans cells response to H5 Ag shows that they migrate from the epidermis and changes in morphology upon its activation. The experiment also indicates that H5 Ag can be successfully delivered to human skin via microneedles. Thus, the results have demonstrated that MNs can create transient microconduits in the skin to allow DNA delivery which results in gene expression in the epidermis layer.

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Obesity of bones: is there a regulatory role for the BK channel?

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Over the past decade, the prevalence of obesity and osteoporosis has increased dramatically. Not only do these two conditions cause significant mortality and morbidity, they share a common progenitor cell, the adipocyte¹. It has been long recognised that a close relationship exists between bone and fat formation, whereby an increase in marrow adipose tissue is accompanied by decrease in osteoblasts proliferation and activity¹. This has been observed in multiple conditions that result in osteopenia including osteoporosis, glucocorticoid treatment, and immobilisation¹. Increasing evidence suggests a role for potassium channels in adipogenesis. Potassium channels thought to be involved are K_{Ca} (Ca^{2+} sensitive) and K_{ATP} (ATP sensitive)². This research will utilise the 7F2 cell line that is osteoblast in nature but can be induced to become adipogenic. These experiments aim to determine whether BK channel is involved in regulating the differentiation of osteoblasts into adipocytes and investigate the differences of the BK channel subunit expression between undifferentiated and adipocytic 7F2 cells and the presence of active ion channels.

RT-PCR (reverse transcriptase polymerase chain reaction) was employed to determine the mRNA expression of the BK channels subunits in both adipocytic and undifferentiated 7F2 cells. Primers specific to the mouse genome were designed to explore the presence of the α subunit and all four (1-4) β subunits. Adipogenesis assays were performed in order to test the effects of the BK channel ligands using various concentrations: TEA (0.3-30 mM), TPA (3-100 μ M), TBA (0.1-2 mM), THA (1-20 μ M), tetrandrine (1-30 μ M), iberiotoxin (10-300 nM), paxilline (0.03-1 μ M) and NS1619 (0.3-10 μ M). The assay was constructed using a conventional adipogenesis medium containing α -MEM, FBS (10%), penicillin/ streptomycin (1%), indomethacin (50 μ M), ascorbic acid (50 μ g/ml) and dexamethasone (10^{-7} M). This assay was quantified on day 5 using Oil Red O staining (for lipid content) and measuring absorbance at 490 nm. Additional adipogenesis assays were performed in order to help further characterise the role of BK in time course assays of days 3, 7 and 8 using (TEA 3 mM), TEA (10 mM), tetrandrine (10 μ M), iberiotoxin (300 nM) and NS1619 (10 μ M). MTS assays were performed in order to test the effects of the following on cell proliferation: (TEA 3 mM), TEA (10 mM), tetrandrine (10 μ M) and iberiotoxin (300 nM). The assay was quantified on day 3 using MTS/PMS and measuring absorbance at 490 nm. Patch clamping was undertaken in order to look for active ion channels in both adipocytic and undifferentiated 7F2 cells. Recordings were made in the cell-attached and inside-out configuration using sodium chloride Locke solution in the bath and high K^+ solution in the electrode.

RT-PCR (at 35 cycles) indicated that all BK channel subunits are expressed at mRNA level in the undifferentiated 7F2 cell but not in the adipocytic cells. Adipogenesis assays demonstrated that TEA (3 mM) and tetrandrine (10 μ M) significantly reduced adipogenesis and TEA (10 mM) significantly increased adipogenesis (compared with a conventional adipogenic medium control) on days 5, 7, and 8 with selective BK channel ligands iberiotoxin and NS1619 failing to demonstrate significant effects on days 3, 7 and 8. Interestingly, MTS assays demonstrated that TEA (3 mM) and tetrandrine (10 μ M) significantly increased cell proliferation and TEA (10 mM) significantly decreased proliferation (compared with a conventional adipogenic medium control). Patch clamping failed to identify the presence of BK channel in either cell types. In adipocytic cells seal success rate was 11/12. Unfortunately they failed to demonstrate any channel activity. 7/12 successful seals were obtained with the undifferentiated cells with active channels evident in two patches. The channels had a unitary conductance of 35pS therefore it is possible that the channel could be intermediate conductance calcium activated channel (IK) or K_{ATP} .

In conclusion, the role of BK in controlling the transdifferentiation potential of osteoblasts into adipocytes is not clear. Evidence for its role in transdifferentiation is; mRNA BK subunit expression differs with cell type, TEA 10 mM increased adipogenesis and decreased proliferation, TEA 3 mM and tetrandrine 10 μ M decreased adipogenesis and increased proliferation. Evidence against its role in transdifferentiation; the BK channel is not present in adipocytic and undifferentiated 7F2 cells and selective BK channel ligands iberiotoxin, paxilline and NS1619 failed to demonstrate a significant effect. It is interesting that TEA 10 mM induced adipogenesis whereas TEA 3 mM decreased adipogenesis. This suggests that the effect is concentration dependant. It is disappointing that BK channel were not evident in either cell type. The mRNA subunit expression suggests that they are present; however protein assays (such as Western blots and immunocytochemistry) would be required in order to establish whether the messenger is translated. Further experimentation should include; QPCR to determine the relative amounts of each subunit, characterise the 35pS channel and protein assays to determine translation of BK subunit mRNA into functional proteins.

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Prostate cancer therapeutics: synthesis of novel oxadiazole-derived CYP24 inhibitors

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Adenocarcinoma of the prostate is the most prevalent cancer in males in the United Kingdom, and causes the second-highest number of cancer-related deaths (behind lung cancer).¹ Treatment at this time usually involves the reduction of serum concentrations of androgens such as testosterone to 'castrate levels' to reduce prostatic tumour growth.² This can be carried out through surgical removal of the prostate or testicles, radiation therapy or through administration of 'anti-androgens', drugs that mimic hormones involved in the androgen synthesis pathway.² However, despite treatment being reasonably successful, the incidence of prostate cancer is thought to be on the increase.¹ This reversal in fortunes may be due to a phenomenon known as 'hormone-refractory prostate cancer' – although androgen levels are seen to be reduced to 'castrate levels', biological markers of prostate cancer such as prostate specific antigen (PSA) are seen to be rising to or at levels diagnostic of further tumour growth.³ One potential therapeutic option that has arisen is the use of vitamin D and its metabolite calcitriol ($1\alpha,25(\text{OH})_2\text{D}_3$), shown to have anti-proliferative and tumour suppressing properties through interaction with the nuclear vitamin D receptor (VDR)⁴ – however, administration of these is impeded by quick metabolism via the CYP24A1 enzyme, which can be abnormally up-regulated by tumour cells.⁴ Therefore, inhibitors of this enzyme structurally based on calcitriol are a viable therapeutic option.

Previously, imidazole-containing derivatives of vitamin D had been synthesised,⁵ which were thought to bind to the enzyme through an interaction between the imidazole nitrogen atom and the charged haem atom of the enzyme's active site. Therefore, it was suggested that increasing the number of nitrogens available for binding may yield an improvement – an oxadiazole ring structure (containing 2 nitrogens) was chosen as a good replacement. The starting material was Boc-protected glycine, which was hydrazinated and then reacted with CS_2 and KOH to form an oxadiazole ring structure bonded to a sulphur group. S-alkylation was carried out with three different brominated organic compounds to provide novelty and variation. Finally the Boc protecting group was removed (acidic conditions) so that the amine could be coupled with a synthesised styryl derivative to give the final compounds.

Through use of analytical techniques (NMR, microanalysis, mass spectrometry) it was shown that the correct intermediates and final compounds were synthesised during the time spent, showing that the reaction scheme chosen was appropriate. However, purity remained a problem with some reactions, and some poor yields were obtained for some compounds; for example, the final compounds provided less than 50mg each at the time of completion, but these samples were ensured to be as pure as possible through recrystallisation (with ethanol/methanol), extraction and column chromatography.

Both positive and negative outcomes were seen at conclusion of the synthesis and analysis. Although yields of the desired final compounds were poor to average, enough could be sent for testing. Inhibitory activity versus the CYP24 enzyme will be evaluated by Prof. Glen Jones, Queens University, Canada. Anti-proliferative activity and other effects on VDR genes in hormone-refractory prostate cancer cell lines will be evaluated by Dr. Paul Thompson at the University of Ulster. Also, since there has been shown to be some value for CYP24 inhibitor therapy in other cancers, samples of our final compounds will be tested for anti-proliferative effects against patient-derived leukaemia cells by Chris Pepper at the University Hospital of Wales, Cardiff. In all cases, results were not available at the time of write-up. Overall, however, the issue of yield is one that must be optimised, especially if one or more of these compounds is proved efficacious and more commercial synthesis will be necessary.

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The effects of long-term prescription drugs and conditioned media on osteoblast and breast cancer cells: an *in vitro* study

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Osteoporosis and breast cancer are major health concerns in the UK. Osteoporosis occurs due to low bone mass and strength.¹ This is caused by an imbalance in bone resorption and bone renewal that can result in fractures. HRT is used to reduce the risk of osteoporosis and therefore has a positive impact on bone.² Breast cancer tumours contain Oestrogen Receptors (ER). Oestrogen binds to these receptors to form a conformational change in the receptor, which favours the recruitment of proteins that promote transcription³ and abnormal cell growth.⁴ HRT leads to prolonged exposure to oestrogens and therefore has a negative impact on breast cancer by increasing risk. This shows that drugs can have varying effects on different cell-lines, therefore this study investigates the effects of long-term use prescription drugs on osteoblast and breast cancer cells in relation to cell number, viability and proliferation of differing cell-lines.

Prescription drugs included fluoxetine (1-100 μ M), gabapentin (1-100 μ M), flurazepam (1-100 μ M), nifedipine (1-300 μ M) and estradiol (1-100 μ M). Cell-lines included MG-63 human osteoblast-like cells, HOB primary human osteoblast cells and MCF7 breast cancer cells. MG-63 conditioned media (CM), HOB CM and MCF7 CM were also used to determine any cross-talk between cell-lines. Growth (cell number) assays were performed in 6 well plates and cell counting, using a haemocytometer, determined the number of total and viable cells present within wells after 5 days. Trypan blue stain also determined cell viability. MTS assays were performed in 96 well plates to determine cell proliferation of all prescription drugs or CM on the cell-lines after 3 days.

The results of Growth and MTS assays can be summarised as follows: Fluoxetine significantly reduced cell number at concentrations of 30 μ M and 100 μ M in MG-63 cells and at 100 μ M in HOB cells. Cell viability decreased, indicating fluoxetine exerted toxic effects at these concentrations. Gabapentin significantly increased HOB cell number at all concentrations but conflicting results were found for each assay when using MG-63 cells. Flurazepam produced a significant increase in number at 1 μ M and a significant decrease at 100 μ M using a growth assay but no significant results were determined from MTS assays. Cell number significantly decreased at 100 μ M and 300 μ M nifedipine in MG-63 and MCF7 (also 10 μ M) cells. HOB proliferation decreased but not significantly. MG-63 cell viability remained high, indicating the decrease in cell number was not caused by toxicity. Estradiol slightly increased cell number at low concentrations (1 μ M-10 μ M) but this decreased at higher concentrations. Osteoblast CM experiments appeared to stimulate MCF7 proliferation significantly and vice versa, indicating there is cross-talk between these cell-lines.

In conclusion, most prescription drugs had similar effects on each cell-line, with some having more profound effects on one cell-line. It is likely that these effects occurred by different mechanisms of action but further investigation is needed to conclude the pathways involved. Further research may also be beneficial to establish growth factors involved in producing cross-talk between bone and breast cancer cells and also to establish the long-term effects of exposure to the prescription drugs used.

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Exploring efficient routes for substituted 2-arylbenzothiazole synthesis as potential antitumour and PET imaging agents

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2-arylbenzothiazoles such as 5F203 and PiB have demonstrated significant potential as selective, potent antitumour and imaging agents for amyloid plaques in Alzheimer's disease (AD) respectively ^{1, 2}. Synthetic routes that afford these pharmacologically valuable compounds are available however they are associated with limitations such as harsh reaction conditions, tedious work up procedures and multi-step transformations to the required precursors³. Two novel methods that may represent more efficient routes for 2-arylbenzothiazole synthesis than those currently available were developed.

Method 1: Copper iodide catalyzed coupling of thiobenzamide to 4-iodotoluene, to generate an intermediate, which can then undergo iodine-promoted cyclization. This was carried out under various reaction conditions. Method 2: synthesis of 2-aminothiophenol and their disulfide counterparts through base hydrolysis of commercially available 2-aminobenzothiazoles, followed by their reaction with substituted benzaldehydes in the presence sodium metabisulphite.

Method 1 proved to be unsuccessful, possibly due to the formation of inactive copper complexes. 6-fluoro-2-(4-nitrophenyl) benzothiazole (SV05) and 6-fluoro-2-(3,4-dimethoxyphenyl) benzothiazole (SV06) were synthesised using method 2 in relatively good yield.

Method 2 which represents a novel method for the synthesis of 2-arylbenzothiazoles may be used to synthesise 2-arylbenzothiazoles with known antitumour activity. This method is being utilised to extend the library of novel 2-arylbenzothiazoles which may then be put forward for cell-line assays to determine whether they have biological activity. There is also potential for these compounds to be used in PET imaging for AD, especially those compounds with fluorine groups.

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The role of L1CAM in the migratory phenotype of Faslodex resistant breast cancer cells

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Breast cancer is the most common cancer in the UK with 45,500 women being diagnosed each year¹. Fulvestrant (Faslodex) is a steroidal, pure anti-oestrogen indicated to treat post-menopausal patients with ER+ breast cancer that has progressed despite initial anti-oestrogen treatment. However, patients can relapse whilst on fulvestrant therapy due to development of fulvestrant resistance. *In vitro* cell models of acquired fulvestrant resistance show an aggressive behaviour (increased invasion and migration). Faslodex resistant (Fas-R) cells have changes in protein expression vs. MCF7 cells. L1 type cell adhesion molecule (L1CAM) is up regulated in Fas-R cells. It has been linked with migration in other cancers such as colorectal and ovarian². L1CAM is implicated in the process of epithelial-mesenchymal transition (EMT). This process involves cells losing epithelial polarity and transforming into single motile cells that are capable of migration³. The aim of this project was to determine the functional relevance of L1CAM in Fas-R cells with respect to migration.

MCF7 cells were routinely cultured in RPMI media and Fas-R cells were derived by continuous exposure to fulvestrant. To investigate the function of L1CAM in Fas-R cells, L1CAM expression was suppressed using siRNA. L1CAM knockout and the resultant changes in protein expression were confirmed using Western blotting; briefly, cells were lysed and proteins resolved using SDS-PAGE before transfer to a nitrocellulose membrane. Immunoprobings were carried out with primary and secondary antibodies. Chemiluminescence was used to detect specific proteins. Immunofluorescence imaging was used to confirm Western results and examine the location of key proteins within the cell and was performed by incubating formaldehyde-fixed cells with primary and fluorescently-tagged secondary antibodies before being counterstained with DAPI and mounted. Proteins were visualised with a fluorescent microscope. Cell-matrix adhesion assays were carried out by coating the wells of a 96-well plate with fibronectin, vitronectin or laminin before adding siRNA-treated Fas-R cells to them. After incubation for two hours, wells were gently washed and adherent cells stained. Numbers of cells adhering to the well floor were then quantified colorimetrically. The ability of siRNA-treated Fas-R cells to migrate over fibronectin was determined using a modified Boyden chamber assay.

Targeted siRNA treatment was shown to reduce L1CAM expression in Fas-R cells. Fas-R cells with reduced L1CAM were significantly less migratory than those expressing the protein ($p < 0.0001$). Reduction of L1CAM was shown to have no effect on the adhesion of Fas-R cells. Fas-R cells with reduced expression of L1CAM also showed a reduction in pMAPK. Immunofluorescence microscopy confirmed this finding and showed the presence of nuclear pMAPK in the Fas-R cells expressing L1CAM. Both Fas-R cells with knock out L1CAM and wtMCF7 cells had diffuse staining indicating cytoplasmic pMAPK with no evidence of nuclear pMAPK. E-cadherin is responsible for maintaining cell-cell contacts and is a tumour suppressor molecule that inhibits migration by suppressing EMT³. Fas-R cells with reduced L1CAM showed increased levels of E-cadherin. Focal Adhesion Kinase (FAK) can be phosphorylated at different tyrosine residues in its structure. FAK Y861 and Y576 have been linked to migration of cells and both were found to be reduced in cells with reduced L1CAM⁴. FAK Y397, which has been linked with adhesion rather than migration, was unchanged in samples with reduced L1CAM supporting the findings of the adhesion assay.

Migration of Fas-R cells is decreased when L1CAM expression is inhibited whereas adhesion was unchanged. This is surprising as adhesion plays a central role in the migratory process⁴. However, the site specific changes in FAK expression support these findings and suggest that L1CAM might interact with FAK to promote migration in Fas-R cells through regulation of FAK Y861. The presence of nuclear pMAPK in Fas-R compared to MCF7 cells suggests that the location of pMAPK is changed when cells develop Faslodex resistance and that inhibition of L1CAM suppresses these changes; moreover, nuclear pMAPK may contribute to the increased migration. L1CAM may lead to migratory behaviour through its interaction with E-cadherin. Over expression of L1CAM in Fas-R cells may lead to a reduction in E-cadherin. This reduction could facilitate breakdown of the cadherin containing cell-cell junctions allowing EMT and hence promoting cell scattering and migration³. In conclusion, L1CAM may be an important novel target for treatment of aggressive endocrine-resistant breast cancer.

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The effect of hydrolysis on the performance of molecularly imprinted polymers

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Molecular Recognition is the specific recognition and interaction of one molecule by another¹. One approach to producing synthetic molecular recognition elements is molecular imprinting². A target molecule serves as the template and forms solution complexes through bond formations with functional monomers. Cross-linkers then fix functional monomers into position. On template removal, binding sites that are functionally complementary to the template are revealed. The resulting matrix is termed a Molecular Imprinted Polymer (MIP). MIPs have proven to be cost effective, simple to synthesise and have found applications as catalysts³, biosensors⁴ and in immunoassays⁵. However MIP technology generally has limited use due to poor overall binding. Modifications to cross-linkers have been hypothesised to increase accessibility & flexibility of MIP binding sites leading to increased selective binding in the MIP. The aims of this study were to investigate the effect of post-polymerisation hydrolysis by synthesising molecularly imprinted and non-imprinted polymer (NIP) microspheres for (R,S)-propranolol by precipitation polymerisation and by varying cross-linker ratios of Divinylbenzene (DVB, a conventional cross-linker) & Ethylene Glycol Dimethacrylate (EGDMA, that introduces hydrolysable bonds) then subjecting polymers to hydrolysis under different conditions.

Polymers containing 20%, 40% and 60% EGDMA were prepared by precipitation polymerisation. Polymers were hydrolysed with 3.56M Potassium Hydroxide in 2:1(v/v) methanol/water at both 60°C and under reflux (80 °C) for 16 hours. Assessment of morphology and binding performance of the varying % EGDMA polymers pre- and post-hydrolysis was via fluorescence microscopy and equilibrium binding studies.

Binding studies demonstrated that the MIPs containing 20% EGDMA bound more the NIPs ($p < 0.05$) and hydrolysis increased binding in the MIPs. MIPs containing higher concentration of EGDMA in most cases did not bind more than the NIP and hydrolysis increased non specific binding. Microscopy indicated that MIPs were larger than NIPs and polymers containing lower proportions of EGDMA were larger than those with higher proportions. Microscopy also indicated that hydrolysis affected NIP morphology more than MIP and heating at 80 °C hydrolysis had a bigger impact on morphology than 60°C hydrolysis.

The deviation in size and shape of the polymer particles was the result of variation in solubility of the growing polymer chains as the proportion of EGDMA was varied. The presence of the template also affected growing polymer solubility resulting in variation in MIP / NIP pairs due to the presence of imprinted sites within the MIPs. This is evidence for a degree of ordering within the MIP and a more hydrophobic polymer. Binding studies demonstrated hydrolysis of MIPs increased binding to propranolol. This implies that by removing bonds, template accessibility to deeply located but well defined binding sites is increased. This investigation showed that by applying a hydrolysis technique to imprinted polymers made with varying ratios of hydrolysable cross-linkers, the binding efficacy of MIPs may be improved. However a balance must exist in hydrolysis extent, between opening up the backbone to improve flexibility and/or accessibility of binding site to improve specific binding and causing the structural break down of the polymer which exposes more non-specific binding sites.

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Competition between absorption and evaporation in the uptake of fragrance materials into the skin

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Fragrances are a mixture of ingredients that are topically applied to the skin to produce a pleasant aroma. This aroma gradually changes over time, decreasing in intensity due to a combination of evaporation into the surrounding area and absorption into the skin. Understanding this evaporation/absorption process is important for two reasons: 'it can aid in the design and evaluation of fine fragrances and other fragranced products' and; 'it can aid in the risk assessment of these products with both skin sensitisation and systemic exposure.'^[1] *In-vitro* experiments are typically carried out in order to determine the evaporation that occurs from dosing of a fragrance material onto the surface of the skin. However, these experiments can prove to be time consuming and expensive to complete and therefore, a theoretically based mathematical model has been developed by Kasting. Comparisons between theoretical data produced using Kasting's equations and existing experimental data for the same fragrance materials was made. The role of the fragrance vehicle in the evaporation and absorption processes was also investigated.

The physical properties of the fragrance materials were found and entered into a Microsoft Excel spreadsheet. Formulae provided for model 1 in the paper 'A physico-chemical properties based model for estimating evaporation and absorption rates of perfumes from skin' by G. B. Kasting and P. Saiyasombati^[2] were then used to calculate theoretical values for evaporation using the literature physical properties. The values obtained were calculated for specific time points and a time-course graph plotted. This was compared with experimental evaporation values from a PTFE sheet where there was no competitive absorption.

Results showed that the experimental fragrance material which had the most similar percentage recovery profile to the theoretical data was dimyrcetol in ethanol. The least similar profile was shown for geranyl nitrile^[3] as experimentally there was still recovery taking place at 24 hours whereas the theoretical evaporation was complete by 6 hours. It was also demonstrated that the vehicle in which the fragrance material was diluted significantly influenced the extent of permeation. Petroleum vehicles resulted in highest permeation, with dipropylene glycol giving the least permeation.

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Synthesis of 2-arylindoles as potential antitumour agents

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Drug resistance is a major problem with anticancer therapies, with multi-drug resistance being a common cause for treatment failure.¹ Novel drugs, acting by an alternative mechanism of action could be beneficial in overcoming problems with resistance.¹ This was highlighted by the discovery of the antitumour benzothiazoles and their development through structure activity relationships (SAR).² Antitumour benzothiazoles are advantageous as they display potent anticancer properties independent of the oestrogen receptor status; with GI_{50} values $<0.1nM$ in MCF-7 (ER+) and MDA 468 (ER-) breast cancer cell lines.² These agents have been shown to mediate their effects through activation of the arylhydrocarbon receptor (AhR), which induces CYP1A1, leading to the formation of active metabolites.¹ However, the physicochemical parameters of the antitumour benzothiazoles are poor, limiting their clinical applications.³ The benzimidazoles were synthesised in an attempt to overcome this issue.³ SAR of the benzimidazoles highlighted that the incorporation of a sulfonyl N-substituted group improved antitumour activity compared to the free benzimidazole ($GI_{50} = 6.82\mu M$). In addition to the developments of the benzothiazole structure, indole containing compounds have displayed anticancer properties as tubulin polymerization inhibitors.⁴ The aim was to synthesise 2-arylindoles and a series of N-substituted analogues, to allow a SAR to be constructed to evaluate their antitumour potential.

The unsubstituted indoles, 2-(3,4,5-trimethoxyphenyl)-1H-indole and 5-fluoro-2-(3,4,5-trimethoxyphenyl)-1H-indole, were initially synthesised. A traditional two step Fischer indole synthesis was used, with an acidic catalyst; using methods reported in literature. Glacial acetic acid was used as the catalyst to synthesise the arylhydrazones from (4-fluoro)phenylhydrazine and 3,4,5-trimethoxyacetophenone. Polyphosphoric acid (PPA) was used as the catalyst for cyclization of the arylhydrazone. The N-substituted indoles were synthesised under inert conditions in anhydrous DMF. Sodium hydride (NaH) was used to increase the basicity of the indole NH. Arylsulfonyl, alkylsulfonyl and methyl N-substituted indoles were synthesised. ¹H-NMR and ¹³C-NMR was used to confirm product purity. Product purification was performed by column chromatography and by recrystallization.

A series of 2-arylindoles were synthesised, with the unsubstituted indoles being produced in suitable yields. A number of problems were encountered with the purification of the substituted indoles. Therefore the pure products could not be isolated within the timescale, in a number of cases.

Product purification was achieved by column chromatography. However, the substituted indoles were generally more difficult to purify than the unsubstituted indoles. A number of anomalies were observed during NMR analysis with some of the N-substituted indoles, possibly accounting for an indole β -substitution.⁵ The syntheses would need to be repeated in order to fully explain this effect and to obtain the pure products in a higher yield. Antitumour evaluation is required for the pure compounds obtained in order for a direct comparison to be made with other antitumour agents and to direct further SAR studies of potential antitumour agents. Physicochemical and biological tests are also required to determine the cellular mechanism action of these agents, prior to clinical development.

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Effects of β -phenylethylamine and tyramine on ileum, colon and trachea

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Trace amines (TAs) are endogenous amines that can be found in the mammalian CNS¹, including β -phenylethylamine (β PEA) and tyramine, which are structurally related to the classic biogenic amines. They can be generated in the body by decarboxylation of their specific amino acid precursors⁴, or obtained from food, like chocolate, wine and cheese². TAs have been known as indirectly-acting sympathomimetic amines (ISA), in which sympathetic effects are induced by releasing the catecholamines, e.g. noradrenaline²; and to generate effects by interacting with other receptors, like 5HT receptors, as well as trace amines receptors (TAAR). The present study determines the contractility effects of β PEA and tyramine on ileum, colon and trachea, thus aiming to identify which mechanisms of actions they utilise to generate effects and identifying any potential TAAR responses.

The isolated ileum and colon were electrically stimulated, which would help to identify any inhibitory effects in response to β PEA and tyramine. The trachea was isolated, cut into a spiral and non-stimulated. All drugs were added cumulatively in half Log increment of molar concentration into the organ bath, except 5HT which was added as single doses. Antagonists were added at final bath concentration of 10^{-6} M and incubated for 15 minutes before re-challenged with the drug. Methacholine was always tested first to assess sensitivity of the tissue. Fresh preparations were made to test different TAs. The changes in baseline and heights of twitches were recorded and expressed as grams of tension and % of inhibition, respectively. Data were presented as means \pm SEM. Student's t test was used to compare paired or unpaired data. The values were considered as significant if $P < 0.05$. Dose ratios were calculated to determine the number of folds the response curves had shifted.

β PEA contracted the ileum, an effect not modified by adrenergic antagonists, thus not sympathomimetic. It was abolished by ritanserin, while relaxation occurred in the colon. In the colon, relaxation was abolished in the presence of adrenergic antagonists and revealed a contractile response, which was maintained in the combination with ritanserin. The residual contraction was independent of adrenergic and 5HT receptors, thus proposed as TAAR effect. Tyramine generated an initial contraction in the ileum, which was absent in the colon. This was abolished by the adrenergic antagonists. Relaxation and inhibition were observed in all preparations. The addition of adrenergic antagonists reduced the effects, and when combined with ritanserin, the effects had merely reduced. The residual effects were independent of adrenergic and 5HT receptors, and thus proposed as TAAR effects. In contrast, the concussed rats were generally less sensitive and only generated small responses. Tyramine relaxed and generated inhibitory effects on both ileum and colon, which were reduced by the adrenergic antagonists. The residual effects could potentially be TAAR effects. β PEA contracted the ileum and was independent of adrenergic receptors. β PEA relaxed the colon but independent of adrenergic antagonists, which could potentially be a TAAR effect. The trachea had moderate contracted in response to methacholine, and merely reduced the baseline at high concentrations of β PEA. No reductions in baseline were observed if tyramine was tested before β PEA.

The present study had shown that β PEA and tyramine have mixed mechanisms of actions; ISA² and interaction with receptors, including TAAR. Contraction was the predominant effect of β PEA on the ileum, which may be mediated by 5HT₂ receptors. The contraction was not observed with tyramine. This suggested the β PEA-containing foods may have the ability to stimulate the gut and to cause diarrhoea. This supports the reason to serve chocolate and cheese after dinner and the probiotics, which claim to aid digestion and relieve bloating³. Therefore, the amount of TAs that we consume and produce in the body may become one of the causative or preventive factors for irritated bowel syndrome (IBS). However, species differentiation must be considered when apply the results of present study into human health. Finally, poor peripheral responses in the trachea may reflect the absence of TAAR in trachea or that the TAAR do not respond to β PEA and tyramine.

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Optimisation of cell-based methods to study the entry inhibition of vaccinia virus

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Vaccinia virus (VACV) is regarded as the prototype orthopoxvirus, commonly known for its previous use as the human smallpox vaccine and its current role in biomedical research¹. A recombinant form of VACV expressing firefly luciferase has been constructed to facilitate the study of viral uptake into mammalian cells. When used in conjunction with small interfering RNA (siRNA), this system may help clarify the antiviral mechanism of action of di-deoxy bicyclic pyrimidine nucleoside analogues (ddBCNAs).² The aims of the present study were to a) confirm the inhibitory effect of the ddBCNA, Cf 2642, on VACV entry into cells, b) to investigate the effects of different siRNA transfection complex components on VACV entry and c) to optimise the methods by which these early experiments are conducted.

Recombinant VACV (v3) was propagated in HeLa cells and titrated to determine the optimum conditions for virus collection. Experimental infection was optimised to achieve the greatest possible luminescent signal by adjusting the multiplicity of infection (MOI), collection time, HeLa cell strain and density, post-transfection infection time-period and well plate format. Two strains of HeLa cell were transfected with either green fluorescent protein (GFP) siRNA, the liposomal transfection agent Oligofectamine™, or a complex of the two and then infected with v3. Luminescence produced by reacting infected cell lysates with firefly luciferase substrate was quantified using a luminometer, and values were later adjusted according to the protein content measured in each lysate.

It was found that the v3 pellet supernatant collected 2 days post-infection produced the highest titre virus, and optimum luminescent signals in assays were generated by infecting transfected HeLa_{WSP} cells in a 24-well format at an MOI of 5, collecting 2 hours post-infection. Interestingly, different HeLa strains showed significant inter-laboratory variation in characteristics, including their propensity to uptake virus at early time-points. We confirmed the inhibitory effect of Cf 2642 on VACV entry into HeLa cells, and found that GFP siRNA complexed to the liposomal transfection reagent Oligofectamine™ also inhibited viral entry. Out of the two components of the transfection complex, Oligofectamine™ was found to be responsible for the viral entry inhibition, as the GFP siRNA demonstrated no intrinsic inhibitory effect. Such effects are postulated to be the product of pro-immune reactions involving endosomal toll like receptors (TLRs)³ or the reported intrinsic off-target toxic effects of the siRNA⁴. However, the relationships involved here remain unclear and further investigation would be required to elucidate the mechanisms responsible for our results.

For more reliable future entry studies involving v3 and siRNA, it is necessary to find a transfection reagent that does not elicit an inhibitory effect on viral entry. Investigations into the inter-laboratory variation of HeLa cells might also be of interest in order to optimise v3 uptake for high titre stock production. Furthermore, methodological modifications that include sonication and gradient purification of crude virus would improve assay reliability and repeatability, and are therefore recommended for future studies.

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