33rd All-Ireland Schools of Pharmacy Research Seminar

The 33rd All-Ireland Schools of Pharmacy Research Seminar took place on the 18th & 19th April 2011 in the Royal College of Surgeons in Ireland. This is an annual research seminar between all of the Schools of Pharmacy on the island of Ireland, including Trinity College Dublin, Queen's University Belfast, the Royal College of Surgeons in Ireland, University College Cork and the University of Ulster Coleraine.

The seminar was formally opened by Professor Hannah McGee, Dean, Faculty of Medicine and Health Sciences, RCSI. The seminar has grown significantly over time and this year there were 18 oral presentations and 67 poster presentations representing all areas of pharmacy research including chemistry, pharmacology, pharmaceutics and pharmacy practice.

The seminar was supported by the following sponsors: Leo Laboratories Ltd, Athlone Laboratories Ltd, GlaxoSmithKline, The Irish Pharmaceutical Healthcare Association, The Irish Pharmacy Union, Clonmel Healthcare Ltd, American Association of Pharmaceutical Scientists and Warner Chilcott.

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Selected abstracts from the 33rd All-Ireland Schools of Pharmacy Research Seminar

Novel Phosphorescent Chromophores for Assessing Protein and other Biomolecules Location and Function

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Apoptosis, or programmed cell death is an evolutionary conserved and highly regulated process that is the principal mechanism for the elimination of aged, damaged, and unnecessary cells. One of the essential characteristic of cancer is the capability to elude or ignore physiologic cues that would initiate this form of cellular suicide in normal cells. This is often achieved through deregulation of apoptotic signalling pathway.

The B-cell lymphoma 2 (Bcl-2) family of proteins is composed of both pro-apoptotic (pro death) and anti-apoptotic (pro survival) members. These proteins maintain the balance between newly forming cells and old dying ones. Through a complex series of protein –protein interactions they mediate the intrinsic and extrinsic apoptotic pathways. The pro death proteins propagate the death signal by inducing permabilization of the mitochondrial membrane, release of cytochrome C, and the activation of a group of intercellular cysteine proteases called capases. Pro survival Bcl-2 family members exert their protective effects by directly binding to and deactivating their pro death counterparts. When anti-apoptotic Bcl-2 family members are over expressed, the ratio of pro- and anti apoptotic Bcl-2 family members is disturbed and apoptotic cell death may be prevented. Cancer cells frequently over express the pro survival Bcl-2 family members to suppress the apoptotic signal in order to promote survival or confer resistance to chemotherapy. Inhibition of these anti apoptotic Bcl 2 Family members offers an attractive target for anti cancer therapeutics.[1]

In this contribution we report on the synthetic strategies to label a pro apoptotic protein, BID, and a small organic mimic that has strong binding affinities to anti apoptotic members, to indentify functional domains and binding partners, respectively. These studies are using ruthenium polypyridyl complexes with unique photophysical characteristics which make them potentially invaluable as probes for such applications. They are long lived, exhibit polarized luminescence, have good photostability, red emission wavelengths, large stokes shifts and oxygen sensitivity. Such chromophores provide unique opportunities for imaging dynamic environmental changes in living cells avoiding limitations associated with fixation. The synthetic strategy, spectral and photophysical properties of metal-ligand peptide complexes are described. Studies on their uptake by live cells are also described.

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Formoterol and Salbutamol are Competitively Interacting with Organic Cation Uptake at the Human Alveolar Epithelial Barrier

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Formoterol and salbutamol (albuterol), two β_2-receptor agonists used in the treatment of COPD and asthma as first-line treatment, carry a cationic charge at physiological pH. In this study, we investigated, if organic cation transporter (OCT) and organic carnitine transporter (OCTN) systems are involved in the translocation of the two drugs in human alveolar epithelial cell monolayers (A549).

A549 cells were grown to confluent monolayers for 5 days. Investigations of OCT/N mediated transport were performed using [^{14}C]-tetraethylammonium (TEA, 10 µM), [^{3}H]-acetylcarnitine (5.5 nM, Ac-Car) and the β_2-receptor agonists (both at 500 µM) in Krebs-Ringer bicarbonated buffer (pH 7.4). A549 monolayers were incubated for 30 min (TEA) or 20 min (Ac-Car) at 37°C, respectively. Competitive inhibition studies were also performed in which TEA uptake (0.25 - 2 mM) was studied in the presence and absence of formoterol (500 µM) and salbutamol (1 mM) for 30 min.

The transporter site for TEA and Ac-Car was observed to be temperature-sensitive and time- and concentration-dependent. The involvement of non-saturable as well as saturable transporter sites for both OCT/N probes was noted. K_m values were calculated to be 744.4±248.6 µM (TEA) and 17.7±14.2 µM (Ac-Car), respectively. TEA uptake was significantly inhibited by MPP^+ (11.5% of control), decynium 22 (5.8%) and amantadine (30.1%). Ac-Car uptake was decreased in the presence of L-carnitine (28.3%) and unlabelled TEA (27.1%). However, MPP^+ showed no effect on Ac-Car uptake. Furthermore, TEA uptake was decreased to 15.4% by formoterol and to 54.5% by salbutamol. Neither β_2-receptor agonist significantly attenuated Ac-Car uptake. Eadie-Hofstee transformation of TEA uptake revealed linearity and K_m values of 4.3±3.2 mM and 4.6±0.4 mM in the presence of formoterol and salbutamol, respectively, which was a 5-fold (formoterol) and 3.5-fold (salbutamol) increase compared to control values (848.5±71.9 µM (formoterol) and 1.3±0.4 mM (salbutamol)). However, no significant change in V_{max} was observed. Formoterol inhibited TEA uptake with a K_i 115 µM, whereas a K_i value of 271 µM was obtained for salbutamol.

Our data suggest that organic cation transporters are the key contributors to membrane transport of organic cations at the human alveolar epithelial barrier. β_2 receptor agonists inhibited TEA uptake via OCTs, however, no interaction between the compounds and OCTNs was observed.

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The Culture of Antibiotic Prescribing in General Practice in Ireland - A Qualitative Study

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Antibiotics continue to be prescribed in primary care for respiratory conditions despite contradicting evidence (1). The risk of antimicrobial resistance in an individual is increased for up to 12 months subsequent to a course of antibiotics (2). Patient expectation for an antibiotic has been shown to influence General Practitioners (GPs) in their decision to prescribe antibiotics. Prescribing also encourages future consultations for similar conditions (3). The aim of this study was to explore the views of GPs on antibiotic prescribing practice in Ireland and the reasons why there a gap exists between clinical evidence and every day practice.

A qualitative study of GPs in Ireland using semi-structured interviews. Audio recordings were transcribed verbatim. Thematic analysis was conducted on NVivo 9®. Ethical approval was obtained from Irish College of General Practitioners Research Ethics Committee.

17 GPs were interviewed with varying years of experience from various regions in Ireland. All GPs felt that antibiotics were over-prescribed and that it was a complex issue. The majority felt it was due to the role of the patient within the consultation "often you feel you are

fighting with the patient when you tell them they don't really need an antibiotic." The disparities in public education was a big theme, "somewhere along the line the legend has got out there that sort of every cough, sore throat, sort of sore ear, really one should be taking an antibiotic if it's not getting better." The use of deferred prescriptions for 'just in case' has become increasingly popular. The main justification for their use was to avoid out-of-hours care and the additional cost implications for the patient to re-consult. However some GPs felt this was 'a cop out' in situations when antibiotics were not necessary and the patient was not happy without the prescription.

GPs feel isolated in their battle to educate patients regarding appropriate antibiotic use. This study highlights the need for a national public awareness campaign in all health sectors. Community pharmacists also have a role educating patients about the self-limiting nature of the majority of respiratory illnesses. GPs may feel that issuing a prescription is part of the service that is expected. However, providing a prescription that may not be necessary is potentially sending a mixed message to patients.

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Microbiological Analysis of Novel Antibiotic Loaded Hydrogel for the Treatment of Chronic Wound Infections

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Categorical evidence has been compiled to support the theory that bacteria growing in matrix-enclosed biofilms are a prolific causation of increased healing time in chronic wound ulceration. Formation of biofilm on host tissue carries negative clinical implications, as pathogens experience protection from the action of antimicrobials and host immune responses. The overall aim of this investigation is to assess the effectiveness of mupirocin loaded PVA-Borate hydrogels in the disruption of *S.aureus* biofilm infections via chemical and physical processes.

A 96 well microtiter plate-based method for antimicrobial susceptibility testing of *Staphylococcus aureus* biofilms has been employed to assess the effectiveness of PVA-Borate hydrogels as a novel treatment for chronic wound ulceration through the local delivery of antimicrobials. *S.aureus* type cultures NCTC 8329 and 11940 were grown in Tryptone Soya Broth at 37ºC for 24h. An inoculum of approximately 10^6cfu/ml was prepared for each strain. Optical density (OD_{620}) was used as a measurement of growth. An automatic 96 well microplate reader was used to measure absorbance every hour over a 24h period with incubation at 37ºC and shaking. In vitro drug release was measured using an enhancer cell method and a regenerated cellulose membrane as the barrier for release. Over a period of 24h samples were withdrawn and analyzed using high performance liquid chromatography.

The minimum inhibitory concentration (MIC) for mupirocin was determined to be <0.16mg/L for both *S.aureus* strains. Drug release profiling evaluated over 24h showed that a sufficient amount of mupirocin can be successfully released from an optimum formulation. The efficacy of mupirocin loaded PVA-Borate hydrogels with respect to exposure time has been explained using a modified 96 well microtiter plate-based method.

Pilot studies have shown the potential for this high-throughput system to be used in further investigations of the effectiveness of novel antibiotic loaded hydrogels in the treatment of biofilm infections.

Enhancing Cell Penetration using Surface-modified Nanoparticles: Enhancing Drug Delivery to the Central Nervous System (CNS)

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Treatment of many CNS disorders remains inadequate due to the difficulty in facilitating drug entry to this region, across the blood-brain barrier (BBB). Polymeric Nanoparticles (NPs) have been investigated as a non-invasive method of delivery to multiple organs and tissues including the

central nervous system (CNS) to achieve a targeted and prolonged drug effect, though success has been limited. The aim of this research was to investigate whether surface modification of nanoparticles (NP) with a membrane translocating peptide enhanced their cell penetration.

Loperamide- or coumarin-6-loaded PLGA nanoparticles (PLGA-NP) were formulated using the single emulsion solvent evaporation technique and surface modified with the peptide octa-arginine (R8-NP). Particles were characterised pre- and post modification for particle size, surface charge and drug release. Surface modification was confirmed by proton nuclear magnetic resonance imaging (H^1-NMR).

Cell uptake of particles in a CaCo2 cell line was explored using flow cytometry and fluorescence microscopy. Ability of particles to cross the BBB *in vivo* was investigated in a mouse model using a hot-plate assay. NP suspensions were administered to mice (C57/BL6) using the intranasal route

This study demonstrates that both PLGA-NP and R8-NP were taken up into CaCo2 cells with R8-NP showing the most efficient uptake, over three times greater than that of PLGA-NP. R8-NP treated mice demonstrated statistically significant ($p<0.05$) anti-nociceptive effects compared to loperamide in saline or PLGA-NP in saline.

This study shows that octa-arginine complexation to PLGA nanoparticles is a promising means of enhancing drug transport across cell membranes including the BBB.

An Overview of the Cardiovascular Health of an Irish Population Sample in an Occupational Setting

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Cardiovascular disease (CVD) is the number one cause of death globally, responsible for 17.1 million deaths annually.[1] This study examined the cardiovascular health of an Irish population sample taken from an occupational setting.

Ethical approval for this study was granted by the local clinical ethics committee. Written consent was obtained from all participants. Data were derived from a sample of 311 workers, aged 20-63 years (72% male), mainly skilled IT and administrative staff, recruited in the workplace. All participants received a standard CVD risk assessment including measurement of Blood Pressure (BP) and lipid profile. Ten-year risk of CVD was measured using the SCORE[2] screening tool; high CVD risk was defined as >5% in 10-years.[2] Results are shown in Table 1.

Table 1. Results of CVD risk assessment for the occupational population sample

	n	Mean	Standard Deviation
Age (years)	311	36.07	6.83
Body Mass Index (kg/m^2)	311	26.7	3.8
Systolic BP (mmHg)	309	126	13
Diastolic BP (mmHg)	309	74	9
Resting Pulse (bpm)	309	65	11
Total Cholesterol (mmol/L)	276	4.83	0.92
Triglycerides (mmHg)	267	1.54	0.88
HDL-Cholesterol* (mmol/L)	271	1.22	0.40
LDL-Cholesterol† (mmol/L)	258	2.94	0.91
Blood Glucose (mmol/L)	276	5.07	0.61
SCORE 10-year CVD risk (%)	234	0.71	0.89

* HDL: High-Density Lipoprotein, †LDL: Low-Density Lipoprotein

It was possible to calculate the ten-year CVD risk for 234 participants using the SCORE screening tool. Only 4 participants (1.7%) were classified as high risk (ie. ten-year CVD risk >5%). This may reflect the lower age profile of the occupational population sample when compare to the population as a whole.[3]

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Non-Adherence in Children with Cystic Fibrosis

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Introduction: For children with chronic illnesses, such as cystic fibrosis, often the most effective treatments available are complex and time consuming to administer. Cystic fibrosis treatment involves a multi-faceted approach combining medication with physiotherapy and a high-calorie diet. Nevertheless, although children may be prescribed the most appropriate treatment and given appropriate advice, taking medicines as prescribed can pose a significant challenge to both parents and children and indeed non-adherence rates in children with other chronic diseases has been shown to be as high as 50%(1). The aim of this study is to evaluate adherence to cystic fibrosis management (digestive enzymes, vitamins and physiotherapy) in children and to examine factors associated with different levels of adherence.
Methods: A cross-sectional study involving children and the parents/guardians of children aged 18 or younger, who have a clinical diagnosis of cystic fibrosis is ongoing. To measure non-adherence to therapies a self-report questionnaire (MARS) is being administered to parent/guardians and children aged 11 or older. To explore beliefs about treatments (necessity and concerns) the Beliefs about Medicine Questionnaire (BMQ) is being administered to children aged 11 years or older and their parents/guardians and to investigate health-related quality of life the Cystic Fibrosis Questionnaire-Revised is being administered to parents/guardians and children aged 6 years or older. Additionally the Centre of Epidemiologic Studies Depression Scale (CES-D) is being administered to parents/guardians to assess the potential level of depressive symptoms experienced.
Results: Forty seven children and their parents have been enrolled in the study to date. Physiotherapy had the highest rate of parent-reported non-adherence (22.2%) followed by enzymes (2.9%) and vitamins (2.2%). In a subset of parent and child reported non-adherence, children were 2-4 times more likely to report non-adherence than their parents. The BMQ showed that parents in general perceived a higher necessity for treatments than children. The necessity scores for vitamins were lower than that for enzymes and physiotherapy. There was a low level of concern regarding all the therapies. More than a quarter of parents screened using the CES-D reported clinically elevated levels of depressive symptoms. Relatively high quality of life was reported for all participating children with the average quality of life domain scores being around 70% or above, except for treatment burden which was lower. Recruitment to the study is continuing, with a target sample size of 100 children/parents.
Conclusion: There was a relatively low level of non-adherence observed in the cystic fibrosis patients recruited to date. On completion of the recruitment, further work using prescription refill and prescription issue data will also be used to validate the results. Modelling will also be carried out to identify 'risk factors' associated with poor adherence.

This research was supported by the Research Forum for the Child (Queen's University Belfast)

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Investigating Naturally-Occurring Nano-sized Exosomes in Cancer

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Exosomes are membrane-bound nano-sized (30-90nm) vesicles which are naturally released from cells through the pleiomorphic endosomal system. A limited number of studies, by ourselves (1) and others, suggest that such exosomes may be involved in cell-to-cell communication. Potentially exosomes have a role as autologous delivery systems that could be harness for personalised delivery of therapeutics into secondary cells. Much has yet to be done, however, to determine the relevance of exosomes in cancer and so the potential to exploit them for therapeutic purposes.

Hs578T is a triple-negative breast cancer cell line; Hs578T(i)8 is its significantly more

invasive clonal population (2). The aim of this study was to investigate the existence and release of exosomes from these isogenic cancer cells; evaluating the relative quantities of exosomes released from each population; assessing their interaction with other cell types and relative effects on phenotypic characteristics of such cells.

Using a combination of filtration and centrifugation (3), exosomes were isolated from medium conditioned by Hs578T and $Hs578T_{(i)8}$ cells. Successful isolation was verified by Western blot analysis for the exosomes marker, TSG101. The quantities of exosomes secreted from these cell lines did not differ significantly (n=4; p= 0.46). However, equal quantities of exosomes from these populations conferred very different effects on secondary cells. Specifically, while Hs578T exosomes did not increase the proliferation of SKBR3 cells (proliferation=1.06+/-0.14 fold) when compared to proliferation in the absence of exosomes, $Hs578T_{(i)8}$ exosomes induced a significant (p=0.004) increase in SKBR3 proliferation rate (1.64+/-0.22 fold). Furthermore, unlike Hs578T exosomes, preliminary results indicate that exosomes from $Hs578T_{(i)8}$ cells increase invasion of SKBR3 cells through extracellular matrix (mean increase =16%). This transfer of more aggressive phenotypic characteristic is further supported by our observation that MDA-MB-231-derived exosomes also stimulate a significant increased invasion of SKBR3 cells (mean increase =24%; p<0.002).

In conclusion, these studies support our hypothesis that exosomes from cancer cells are involved in cell-to-cell communication. Here we report exosomes from donor cells cause adverse effects on secondary/recipient cells, reflecting the characteristics of the cells from where they derived. Future studies will access these effects on a broader range of secondary cells, including normal cells. The molecular profile of such exosomes will be investigated using transcriptomics and proteomics approaches, to determine their content as potential biomarkers and possibly also therapeutic targets.

Acknowledgements*:* TCD's Pharmaceutical Research & Development Awards; Science Foundation Ireland for the SRC award to MTCI (08/SRC/B1410); The Marie Keating Foundation PhD Scholarship Award.

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Permeation of Model Drugs from Soluble Polymeric Microneedle-mediated Transdermal Delivery: Potential Application to Paediatric Dosing

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Microneedles are an emerging strategy employed to overcome the skin's *stratum corneum* barrier[1]. In this proof-of-concept study, we determined the percutaneous permeation profiles of two model drugs commonly used in paediatric application, caffeine and lidocaine, across three different *in vitro* skin models. Both drugs were encapsulated in soluble conical-shaped polymeric microneedles with 600 µm in height, 300 µm in base width and 300 µm interspacing. The skin models were dermatomed (300-350 µm) and full thickness (700-750 µm) neonatal porcine skin and a synthetic lipophilic membrane, Silescol® (50 µm). Soluble polymeric microneedles were fabricated from aqueous 20% w/w Gantrez® AN-139 gels by micromoulding[2]. *In vitro* permeation of caffeine and lidocaine from soluble polymeric microneedles was evaluated using Franz diffusion cell studies over 24-hours.

The cumulative percentage of caffeine and lidocaine permeated across dermatomed porcine skin over 24-hours were 58.45±8.60% (n=6) and 39.33±8.92% (n=6) respectively. The cumulative percentage of caffeine and lidocaine permeated across the full thickness porcine skin were 49.55±3.90% (n=6) and 26.13±2.15% (n=6) respectively. The cumulative percentage of caffeine and lidocaine permeated across Silescol® synthetic membranes over 24-hours were 7.87±3.20% (n=6) and 3.28±0.64% (n=6) respectively.

There were statistically significant differences in the cumulative release of both drugs among the three *in vitro* models over 24-hours (in all cases *P* values <0.001). Full thickness porcine skin showed less data variability than dermatomed, possibly because it was less prone to any skin manipulations (e.g. hair removal) during the study, due to its

thickness. The release trend across dermatomed porcine skin followed approximate first order kinetics. This tissue provides lower barrier properties than the full thickness skin due to its relatively thin structure[3]. For this reason, the kinetic parameters of skin permeation obtained may overestimate flux across human skin, as the microneedle tips were directly in contact with the receptor PBS upon application, hence facilitating rapid microneedles dissolution and subsequent drug release *in vitro*. The release profiles across full thickness skin followed an approximate zero order trend that possibly indicates the contribution of the barrier's structures. Accordingly, this model may underestimate flux from the system across human skin. Thorough mathematical modelling is now required to predict drug absorption *in vivo* from this *in vitro* study. Silescol® is not a membrane of choice for this microneedles study, as the pores created by microneedles tended to rapidly close after microneedle dissolution, as observed by microscopy and TEWL studies (data not shown).

This study draws attention to the potential use of soluble polymeric microneedle mediated transdermal drug delivery in paediatric applications as an alternative to conventional drug therapies such as, oral and intravenous. Microneedles are perceived as painless in comparison to hypodermic needles and safe with minimal local reactions in human[4] thus provides a favourable method of drug administration in paediatric. Further work is now on-going in constructing mathematical modelling to conceptualise drug permeation *in* vivo and conducting *in vivo* drugs permeation studies in animal model.

This work was supported by BBSRC grants BB/EO20534/1 and BB/FOF1287 in collaboration with The National University of Malaysia and Malaysian government.

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Evaluation of Numeracy Competency in Pharmacy Students

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Pharmacist's competency in pharmaceutical calculations is essential to ensure patient safety. This is recognized within the syllabus of the National Pharmacy Internship Programme (NPIP) which includes a specific calculations exam (PRE, summative assessment: licensure). Therefore in order to determine whether basic numeracy issues exist within the School of Pharmacy, diagnostic testing was introduced for Junior Cycle students and MPharm interns under the modules JC2 and MP1 respectively. Diagnostic testing is used extensively in universities worldwide to help academics define the nature and extent of any numeracy problems (1, 2). The questions for the assessment were randomly selected from the 1999 and 2003 'Trends in International Mathematics and Science Studies (TIMSS) (3). Junior Cycle students averaged a score of 84% on the test, while MPharm interns attained 93%. These results indicate that the majority of Junior Cycle students and MPharm interns have good numeracy competency. However the numerical performance of the Junior Cycle students and interns was not predictive of their ability to perform pharmaceutical calculations. Based on these results it would appear that a combination of numeracy and a specific mathematical knowledge of pharmaceutical calculations are required to ensure competency in pharmaceutical calculations, with specific teaching of pharmaceutical calculations appearing to be effective at improving student competence.

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Studies in the Synthesis and Impurity Profiling of the Precursors of the Amphetamine Known as Bromo-DragonFLY

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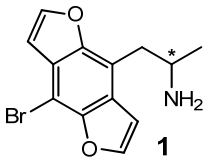
Designer *alpha*-methylphenethylamine drugs of abuse sold on the illicit market can contain high levels of unwanted impurities inherent from the manufacturing process employed by underground laboratories, which rarely incorporates any degree of quality control. Each particular chemical route produces a unique set of impurities known as route-specific markers. These impurities or chemical signatures are of particular interest to the forensic chemist as they can be used as a tool to determine the synthetic protocol. Impurity profiling is used to generate this chemical fingerprint, providing useful information to the drug enforcement agencies, enabling them to monitor underground drug manufacturing and detect and shutdown illegal laboratories. Impurity profiling also facilitates the identification of potentially harmful impurities in drugs.

The work described in this research talk focuses on the synthetic steps leading to the Leuckart synthesis of 1-(8-bromobenzo[1,2-*b*;4,5-*b*']difuran-4-yl)-2-aminopropane **1** (Bromo-DragonFLY) and the impurity profiling of its precursors. The synthesis of the key precursor 1-(2,3,6,7-tetrahydrobenzo[1,2-b:4,5-b′]difuran-4-yl)propan-2-one **2** was adapted from work carried out by Keating (1) and from the literature by Chambers *et al.*(2) and Monte *et al.*(3). This investigation involved a detailed analysis of the chemical route employed for potential impurities and possible synthetic deviations.



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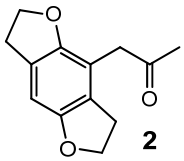

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Carbon Nanotube Surface Modification Increases Blood-Surface Interactions in Cardiopulmonary Bypass

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Introduction: Blood-surface interaction in extracorporeal circuits requires the infusion of a systemic anticoagulant to prevent devastating clot formation. Bleeding and thrombosis are the most common serious complications of extracorporeal circulation(1). Platelet activation upon contact with proteins adsorbed to foreign surfaces is the primary mechanism underlying clot formation(2). The general hypothesis driving our research is that surface modification of the extracorporeal circuit using carbon nanotubes, functionalised with antiplatelet agents, could decrease blood-surface interactions, thus attenuating systemic complications. The aim of the current research was to investigate interactions between non-functionalised, carbon nanotube-modified surfaces of extracorporeal circuits and blood *in vitro* and in a rabbit model of extracorporeal circulation *in vivo*.

Methods: Polyvinylchloride was coated with a surface layer of immobilised multi-walled carbon nanotubes using a swelling and ultrasonification technique. Platelet-surface interaction in a dynamic *in vitro* flow model of circulation was assessed using the Q-Sense nanobalance system. A rabbit model of extracorporeal circulation was used to test blood-surface interaction *in-vivo*.

Results: Significantly increased platelet aggregation was observed in *in-vitro* testing ($n=8$, $p<0.05$). Significantly increased clot formation was observed in *in-vivo* testing ($n=8$, $p<0.05$).

Conclusion: Surface-immobilised multi-walled carbon nanotubes, non-functionalised with antiplatelet drugs, cause platelet aggregation and increased clot formation *in-vitro* and *in-vivo*.

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Investigating the Mechanisms of Docetaxel Resistance in Prostate Cancer

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Prostate cancer is the most commonly diagnosed cancer, excluding basal and squamous cell skin cancer, among males and is the second leading cause of cancer related deaths (1). Docetaxel, at present, is the first line treatment for Hormone Refractory Prostate Cancer (HRPC). Despite the importance of docetaxel in this clinical setting, a major drawback to this treatment is its limited use due to inherent or acquired drug resistance (2,3).

In order to investigate the potential mechanisms of docetaxel resistance, we have developed two docetaxel-resistant *in vitro* cell line models (DU145RD and 22RV1RD) for prostate cancer. The aim of this study was to: (i) characterise these resistant variants in comparison to their respective sensitive parent cells (DU145 and 22RV1): (ii) investigate cross-resistance to other chemotherapeutic drugs; (iii) investigate the relevance of exosomal secretion from these cells, and (iv) investigate the differential-expression of intracellular and extracellular miRNAs in/from DU145, DU145RD, 22RV1 and 22RV1RD that may identify potential mechanisms of docetaxel resistance.

Here docetaxel-treated variants were found to be substantially more resistant to docetaxel than sensitive cells (>70-fold for both DU145RD and 22RV1RD). In addition, resistant variants had distinct motility, migration and invasion capacity. We found that DU145RD cells did not express the P-glycoprotein efflux pump (MDR-1/P-gp) whereas 22RV1RD cells showed some low level expression, suggesting that this efflux pump may be in part, but not wholly, responsible for docetaxel resistance in 22RV1RD. Exosome isolation from DU145RD cells were found to decrease the invasive capabilities of DU145 and 22RV1 cells. Similarly, these exosomes decreased motility of DU145 cells. The current findings of this project will be presented with the discussion of future plans to globally profile the contents (including miRNAs) of DU145 cells, DU145RD cells, 22RV1 cells and 22RV1RD cells and their respective secreted exosomes.

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Prescription Patterns for Patients Admitted from Care Home Settings to an Acute Hospital Setting: A Cross-sectional Study

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Several validated screening tools are available to identify potentially inappropriate prescribing (PIP) in older adults. The most widely used criteria are Beers' criteria [1,2]. However, a limitation of using these criteria is that up to 50% of the drugs listed are unavailable in European formularies [2]. A new screening tool has been developed which incorporates the Screening Tool of Older Persons' Prescriptions (STOPP) to identify cases of PIP, and the Screening Tool to Alert doctors to Right Treatment (START) to identify cases of potential prescribing omissions (PPO) [3] .The aim of this study was to measure the prevalence rates of cases of PIP and PPO, using STOPP and START criteria, for patients admitted from care home settings to hospital.

Clinical and prescription records were reviewed for patients over 65 years of age who were included in an ongoing study. STOPP and START criteria were applied to identify cases of PIP and PPO for each patient on admission, during hospitalization and on discharge. Independent verification was undertaken by a consultant geriatrician. Ethical approval was not required since this study was deemed to be service evaluation.

To date, records for 30 patients have been reviewed. Median patient age was 86 years, and 63.3% of patients were female. The most frequent medical conditions were dementia, hypertension and diabetes mellitus. Half of all patients had a history of falls. The mean number of medicines (± SD) and prevalence of cases of PIP and PPO on

admission, during hospitalization and on discharge are shown in Table 1.

Cases of PIP and PPO were prevalent among the study population. Further data collection will help to indicate if there are significant differences in prevalence of PIP and PPO on admission, during hospitalization and on discharge, and if STOPP and START criteria can be used to judge medication appropriateness for older patients admitted from care homes to hospitals.

Acknowledgments: Ward clerks in participating wards in the hospital and the University of Jordan for sponsorship.

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Table 1. Prevalence of cases of Potential Inappropriate Prescribing (PIP) and Potential Prescribing Omissions (PPO) in the study population

Parameter	Mean (SD) number of medicines prescribed	Mean (SD) number of cases of PIP or PPO per patient	% of patients with one case of PIP or PPO	% of patients with two cases of PIP or PPO	% of patients with three cases of PIP or PPO
On admission	9.83 (3.24)	1.70 (1.44)	43.0%	30.0%	36.7%
During hospitalization	13.33 (4.35)	1.70 (1.46)	13.3%	20.0%	13.3%
On discharge	8.93 (2.82)	1.36 (1.29)	13.3%	10.0%	10.0%

Synthesis and Serotonin Transporter Activity of 1,3-bis(aryl)-2-nitro-1-propenes as a New Class of Anticancer Agents

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Structural derivatives of 4-MTA, an illegal amphetamine analogue have been previously shown to have anticancer effects *in vitro* [1, 2]. The synthesis of a series of novel 1,3-bis(aryl)-2-nitro-1-propene compounds related in structure to 4-MTA and other amphetamine based compounds is presented. A number of these compounds containing a classic nitrostyrene structure are shown to have antiproliferative activities *in vitro* in a range of malignant cell lines, particularly against Burkitt's lymphoma derived cell lines, whilst having no effect on 'normal' peripheral blood mononuclear cells. Such effects appear to be independent of the serotonin transporter, a high affinity target for amphetamines and independent of protein tyrosine phosphatases and tubulin dynamics both of which have been previously associated with nitrostyrene-induced cell death. We demonstrate that a number of these compounds induce caspase activation, PARP cleavage, chromatin condensation and membrane blebbing in a Burkitt's lymphoma-derived cell line, consistent with these compounds inducing apoptosis *in vitro*. Although no specific target has yet been identified for the action of these compounds, the cell death elicited is potent, selective and worthy of further investigation.

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Soluble Polymeric Microneedle Arrays for Enhanced Intradermal Delivery of a Model Antigen

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Skin is considered an attractive site for vaccine delivery, since it has an abundance of antigen presenting cells (APCs). Vaccines are usually delivered across the skin using hypodermic injection, which presents several drawbacks including accidental needle-sticks, pain, and needle phobia. Therefore, new vaccination techniques have been proposed to replace needles and target skin's antigen presenting cells. One of the most promising techniques is minimally invasive polymeric microneedles loaded with antigen which could have a potential to replace hypodermic needles and produce robust immune responses. Soluble polymeric microneedles have several advantages over conventional methods of vaccination as well as other types of microneedles such as solid and coated microneedles. Soluble polymeric microneedles can provide safe, painless, self-administration and quickly dissolve in body fluid leaving behind no biohazardous waste.

The aim of the present work was to determinate the stability of a model antigen (ovalbumin) incorporated into microneedles, after different storage times.

Microneedles were prepared from aqueous blends of 20% w/v poly (methyl vinyl ether/maleic acid) (PMVE/MA) loaded with the model antigen ovalbumin. Ovalbumin stability after being incorporated into microneedles was determined through probing its primary, secondary structure and content, at different storage times using Western blot, circular dichroism and BCA kit.

The results showed that, PMVE/MA has a negligible effect on ovalbumin integrity. This was confirmed by the three different stability assays. Furthermore, Western blot analysis showed no ovalbumin degradation. Ovalbumin, secondary structure remained in its native state since no difference was seen in ovalbumin spectrum after being incorporated into microneedles. Ninety six per cent of the theoretical ovalbumin loading was recovered from dried microneedles.

Soluble polymeric microneedles prepared from aqueous blends of 20% w/v PMVE/MA incorporated with antigen may have a potential in intradermal vaccination due to targeting immune cells in the skin more effectively than conventional hypodermic injections.

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Community Pharmacists' Views on Benzodiazepine Prescribing and Supply

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The Benzodiazepine Committee was established in 2000 to investigate benzodiazepine prescribing and use in Ireland, and to develop good practice guidelines (1). It has been widely documented that benzodiazepines are often inappropriately prescribed and/or misused. However, little research has been published to date on community pharmacists' role in benzodiazepine supply.

The main aim of this study was to assess the attitudes, experiences and knowledge of community pharmacists regarding current benzodiazepine policy and practices (prescribing and dispensing), including benzodiazepine discontinuation. The study consisted of an anonymous, self-administered, postal questionnaire of community pharmacists. The questionnaire was distributed to 857 community pharmacies in Ireland which were selected using a geographically stratified random sampling method. One combined thank you/reminder letter was issued five weeks after the initial distribution. All viable data was coded and entered into PASW Statistics 18.0 for analysis. The response rate was 38%.

It was found that there is a low level of awareness (33%) among community pharmacists of official sources of benzodiazepine guidelines but that the vast majority (93%) agree that such guidelines are relevant to them. Given that a large proportion of pharmacists also agree (47%) that they have a professional responsibility to ensure prescriber adherence with relevant guidelines, increasing community pharmacists' awareness of benzodiazepine guidelines could lead to enhanced enforcement.

Long-term benzodiazepine use, in contravention of prescribing guidelines, was reported to be widespread. However, a positive association was found between the number of patients reported to have undergone gradual dosage reduction and contact by community pharmacist respondents with prescribers about their patients' benzodiazepine use. This highly significant finding ($p = 0.005$, χ^2-test) suggests that in cases where patients undergo reduction/discontinuation of benzodiazepine use, greater levels of active communication between the pharmacist and prescriber may favour a positive clinical outcome.

Community pharmacists agree (77%) that they possess the necessary knowledge and skills to engage with patients on the subject of benzodiazepine use and the vast majority (83%) of them would be willing to play an active role in initiating patients in a benzodiazepine discontinuation/dosage reduction programme. Such a programme is currently being developed by the researchers with a view to exploring and enhancing the role of the community pharmacist in this area.

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Bioavailability of Fenofibrate in an *in vivo* Pig Model using a Lipid-based Formulation Approach

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Introduction: Fenofibrate is a BCS class II molecule with a low aqueous solubility and high logP (5.19) [1]. The bioavailability of fenofibrate in conventional dosage forms is low and variable but increase in the fed state [2]. Therefore it represents a class of drug that should benefit from formulation in a lipid-based drug delivery system [3]. This study was designed to determine the effect of a lipid-based formulation approach on the bioavailability of fenofibrate in a pig model.
Methods: Three prototype formulations were identified by constructing phase diagrams, assessing solubility of fenofibrate in lipid-based excipients and dilution of the formulation in biorelevant media. The prototype formulations consisted of a medium chain and long chain lipid in a self-micro emulsifying drug delivery system (MC-/LC SMEDDS), and, an oil free (surfactant only) system. *In vitro* dispersion and digestion characterisation were also carried out. The formulations were administered orally to pigs in a two way cross over study followed by repeated blood sampling from an indwelling jugular vein catheter at 0, 0.5, 1, 2, 3, 4, 6 8, 12, 24, 48 hours. The plasma samples were analysed by HPLC.
Results: Based on the *in vitro* data the three formulations were characterised according to the Lipid Formulation Classification Scheme [4]. The long and medium chain lipid SMEDDS were classified as Type IIIA formulations. The surfactant only formulation was classified as a Type IV formulation. Evaluation of digestion using the *in vitro* digestion model showed the rate of digestion for MC SMEDDS was faster compared to LC-SMEDDS but Type IV formulation was digestion independent. When administered to conscious fasted pigs the plasma concentration versus time profile was comparable between all three formulations.
Conclusions: A conscious pig model has been developed to determine bioavailability of oral dosage forms. Type IIIA and Type IV formulations were considered to have comparable bioavailability in this model. The differences detected in the *in vitro* digestion studies did not appear to influence bioavailability in the pig model.

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Stabilisation of W/O/W Nanoemulsions Through the use of Stabilising Agents Poly(vinyl alcohol) and Nonionic Surfactants Tween 20 and Span 80

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The formulation of an orally administered peptide drug has been a coveted and elusive design. It was first suggested by Engel *et al.* in 1968 using the water-in-oil-in-water(W/O/W) emulsion

technique employed by Herbert in the development of an antigen adjuvant (1,2). Employing biodegradable polymers as encapsulants allows for shielding of the drug whilst protecting it from the harmful proteases found in the gastrointestinal tract. Emulsions in the nanoscale range are considerably more beneficial than microemulsions as they have the ability to cross tight junctions into cells which had not previously been accessible. The development of such an emulsion has been hampered in part by the inability to produce and maintain a nanoemulsion that can withstand the changes required to produce a stable drug with a relatively long shelf life under standard conditions. Often is the case that large sedimentary particles are formed, a potentially fatal problem for intravenously delivered drugs and for oral drugs a problem that would effectively prevent the therapeutic agent from reaching its desired target (3).

Poly(vinyl alcohol) (PVA) and non-ionic surfactants were utilised in order to stabilise the nanoemulsion thereby reducing the particle size and decrease particle sedimentation by increasing Brownian forces within the emulsion.

A primary emulsion was formed through homogenisation of the internal aqueous phase containing ovalbumin, as a model protein, dissolved in varying concentrations of Tween 20 and the oil phase containing PLGA dissolved in varying concentrations of Span 80 using dichloromethane as the dissolution media. A 2% protein load was observed with a phase ratio volume being 1:4. The primary emulsion underwent homogenisation with 1.25% PVA to form the final w/o/w emulsion. Solvent extraction followed

A similar experiment was conducted without the use of surfactants with the internal phase being replaced by varying concentrations of PVA (0-5% wt) and the oil phase being dichloromethane alone. All other factors remained the same.

It was found that increasing the concentration of Tween 20 at constant concentrations of Span 80 had a positive effect on reducing particle size when compared to PVA. Furthermore, by increasing the concentration of Span 80 a further particle size reduction was observed. This suggests that a reduction in particle size, whilst more dependent on Span concentration, is dictated by both Span 80 and Tween 20. The Hydrophilic-Lipophilic Balance (HLB) achieved was similar to that reached by Khoee and Yaghoobian suggesting that it is the increase in hydrophobicity and Critical Packing Parameter (CPP) that resulted in decreased particle size(4).

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Evaluation of Novel Tubulin Inhibitors as Tumour Vasculature-targeting Agents

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Targeting of tumour angiogenesis and vasculature represents an ideal approach in the design of novel cancer therapeutics since all types of solid tumours are dependent upon a blood supply rich in oxygen and nutrients for growth and survival (1). The best known vascular disrupting agents are the tubulin binding agents, which as a result of their effect on microtubule dynamics; cause cell cycle arrest, morphological changes to endothelial cells and subsequent tumour vascular shutdown (2).

In our laboratory, several classes of novel tubulin inhibitors have been designed, synthesised and investigated as anti-vascular agents in; *in vitro, ex vivo* and *in vivo* settings. Their effect in these assays is being reported here. Using the standard MTT assay, their effect on cell proliferation was evaluated using both human umbilical vein endothelial cells (HUVECs) and human adenocarcinoma prostate cancer cells (PC-3). The effect of test compounds on the cell cycle was performed using flow cytometry while immunofluorescence staining determined their effect on the tubulin cytoskeleton. Change to endothelial cell morphology was studied microscopically following cellular treatment and staining with crystal violet. The *ex vivo* rat aortic ring assay was performed in order to examine the anti-angiogenic/vascular effects of the test compounds, while the PC-3 tumour xenograft model was established to determine the activity of the most potent tumour vasculature targeting agents *in vivo*.

The data obtained to date on our vascular targeting agents has shown that the majority of test compounds; display potent nM level anti-proliferative activity, induce cell cycle arrest at the G_2/M phase, disrupt tubulin cytoskeleton, cause reversible morphological changes to endothelial cells, inhibit angiogenesis, cause rapid microvessel breakdown and in preliminarily *in vivo* studies reduce tumour volume in prostate xenografts.

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The Pursuit of miRNAs as Minimally-invasive Biomarkers Predictive of Response to Anti-cancer Treatment for HER2 Positive Breast Cancer

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The identification of minimally-invasive biomarkers, in efforts to aid in patient-tailored disease management, has become a key focus of cancer research. Recent studies by ourselves and others have reported the existence of circulating miRNAs associated with breast cancer (1-4). Despite this progress, resistance to anti-cancer treatment remains a major obstacle in the treatment of this disease and unfortunately there are no reliable methods of predicting sensitivity/resistance to anti-cancer agents.

This study aimed both to: (i) identify miRNAs differentially-expressed in the breast cancer cell line SKBR3 and its Trastuzumab (Herceptin) resistant variants SKBR3-H which developed resistance by exposure to this drugs for 6 months; and (ii) investigate if corresponding differential levels of miRNAs were detectable in conditioned media (CM) from these cells, reflecting the relative sensitive/resistance profile of the SKBR3-H versus SKBR3 cells.

Global analysis of miRNAs was successfully performed on biological triplicate RNA specimens isolated from SKBR3 cells, SKBR3-H cells and on their respective CM, using TaqMan low density arrays representing 667 human miRNAs. 71.5% (477/667) miRNAs were detected in SKBR3; most (85%; 406/477) of which were detected in its CM. For SKBR3-H, 75.7% (505/667) miRNA analyzed were present in the cells, with 70% (354/505) of these also detected in corresponding CM. Of the 187 miRNAs up-regulated by at least 2-fold in SKBR3-H compared to SKBR3 cells, 6.42% (12/187) were up-regulated in corresponding CM. Of the 62 miRNAs down-regulated by at least 2 fold in SKBR3-H compared to SKBR3 cells, more than half (53.2%; 33/62) were down-regulated in corresponding CM.

Overall, the many hundred (>400) miRNAs detected in CM further supports the existence of extracellular miRNAs and so their potential as minimally-invasive biomarkers. Furthermore, we identified a number of miRNAs that may help predict response to Trastuzumab. Clinical studies on patient sera is now warranted, together with functional analyses to determine the molecular mechanisms associated with these miRNAs and their potential to be exploited as therapeutic targets in breast cancer.

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