PL1
UNDERSTANDING SOLUBILITY OF THE MOLECULE

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A good understanding of a baseline solubility characterization is fundamental in order to guide the selection of formulation approaches and accurate predictions improvements of API solubility and/or dissolution by both chemical (salts and prodrugs) and formulation (i.e. particle size reduction, lipid formulation and cyclodextrine complexation) approach.

Solubility of Active Pharmaceutical Ingredient (API) has always been a concern for formulators, since inadequate aqueous solubility may limit bioavailability of oral products. In recent years, number of low solubility compounds has increased within the pharmaceutical industry pipeline. This challenge has been faced by the pharmaceutical industry by improving knowledge of important physico-chemical characteristic such as solubility, ionization constant and partition coefficient.

Solubility of drugs is influenced by many variables (pH, solid state, presence of counter-ions, temperature, etc.). Even though a single point determination has some value, profiling the solubility of a compound as a function of one more of these variables often yields a wealth of information.

A data fitting approach for determination of pH solubility profile will be presented. Characterization of the solid in equilibrium with different solution media will be discussed.

Biorelevant solubilities will be also taken in consideration through the use of simulated gastro-intestinal fluids. Building on the equilibrium solubility knowledge, these data will be used in order to speculate on a theoretical in-vivo profile to be correlated with drug product performance.

REFERENCES

PL2
IDENTIFICATION AND DEVELOPMENT OF NEW ANTI-CANCER DRUG COMBINATION STRATEGIES

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Advanced forms of cancer are generally treated using chemotherapy drugs. Unfortunately, many tumours are intrinsically resistant or develop this characteristic over the course of treatment, ultimately leading to continued growth and/or metastasis. Optimal distribution and delivery of cancer drugs plays a key role in their efficacy and is very dependent on the pharmacokinetics of the agent at the cellular and whole body levels.

Cellular protein transporters belonging to the ATPase-Binding Cassette (ABC) family have a major role in the physiological transport of many pharmaceutical, particularly anti-cancer drugs. In cancer cells, over expression of these transporters (common for many resistant cancers) can lead to reduced intracellular anti-cancer drug concentrations, abrogating the activity of these agents. However, some of these transporters, especially P-glycoprotein (P-gp, MDR-1, ABCB1), are significantly expressed in the organs of drug elimination and play an important role in the normal pharmacokinetic process of anti-cancer drug clearance, vital for terminating the acute toxicity of such agents. Most small molecules are substrates for one or more of the ABC-transporters. Hence, some established pharmaceuticals may have useful dual roles in cancer pharmacology in combination with existing treatments, while others may seriously augment the toxicity and side effects of anti-cancer drugs. The complexities of this type of endeavor were illustrated, decades ago, where the ability of the cardiovascular drug, verapamil, to inhibit P-gp initially demonstrated therapeutic promise but ultimately failed to provide increased therapeutic efficacy clinically because of unfavourable pharmacological interactions in combination cancer treatment schedules.

Through the use of specific cell models, we have identified that the established Non-Steroidal Anti-Inflammatory (NSAID) agent, sulindac, can inhibit the Multiple Drug Resistance (MRP-1) transporter protein (an ABC transporter related to P-gp). Our research illustrated that this effect was distinct to its anti-inflammatory action and could restore MRP-1-substrate cancer drug-sensitivity in relevant cancer cell models. MRP-1 is an attractive resistance target as it is commonly overexpressed in cancer but does not seem to play a major role in drug elimination.
Through clinical collaboration we have evaluated this laboratory observation in a successful phase I study and the findings have now led to a phase II evaluation of sulinad in combination with chemotherapy in advanced melanoma which is currently underway. Screening of other small molecule agents has illustrated how drug transporter activity can be modulated but careful laboratory evaluation of the underlying mechanism of interaction with the transporter is necessary to evaluate the likely clinical potential of such findings.

Clinical evaluation of the potential of established pharmaceuticals for combination cancer therapeutic application is an attractive drug development path as, typically, the pace of clinical translation of such regimes is much more rapid with a greatly reduced burden of pre-clinical testing required prior to early phase trials.

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REFERENCES

PL3
CRYSTAL STRUCTURE ANALYSIS AS A TOOL IN DRUG INVESTIGATIONS AND DESIGN

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Crystal structure analysis is one of the most important physico-chemical methods of solid state studies and becomes now almost inevitable stage in the course of drug investigations.

This lecture will be focused on the X-ray crystal structure analysis (XCSA), though also neutrons and electrons can be used in a similar way. In XCSA the X-ray beam is diffracted on the electrons belonging to atoms ordered in the crystal. The so-called diffraction patterns, from which the information about the directions and the intensities of the diffracted beams can be extracted, are collected with the use of special equipment called diffractometer and interpreted mathematically. The result is the three-dimensional electron density distribution from which we can read out the atom positions and, at the same time, the geometry of the molecules, the bond lengths and bond angles as well as torsion angles. Also the intermolecular interactions such as hydrogen bonds, van der Waals and π-π, dipol-dipol and others can be localised and characterised.

There are two main types of XCSA, namely the single crystal structure analysis (SCSA) and the polycrystalline or powder crystal structure analysis (PCSA). The first of them is most often used for crystal structure determination, the other is mainly employed as a method of identification, but thanks to recent development of PCSA, it may also replace SCSA in cases, when the single crystals cannot be obtained.

The application of both, SCSA and PCSA will be characterised in treatment of the strictly crystallographic problem of drug polymorphism; the examples of the effects of this phenomenon on solubility and availability of drugs will be given.

The following aspects of SCSA application in drug investigation will be described as the most interesting and important:

− determination of the 3D structure of drug and potential drug molecules with stress on their absolute configuration and conformational preferences as well as the ability to interact with their environment. As examples the antimalarial, anti-HIV drugs and potential drugs, as well as some reactivators of the acetylcholinesterase inhibited by phospho-organic compounds will be given;

− structure determination of protein (especially enzyme) complexes with small molecules; as examples the anticancer and some other drugs will be given.

The application of both, SCSA and PCSA will be characterised in treatment of the strictly crystallographic problem of drug polymorphism; the examples of the effects of this phenomenon on solubility and availability of drugs will be given.
NEW NITROGEN-CONTAINING COMPOUNDS: SYNTHESIS AND BIOLOGICAL ACTIVITY

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We have developed several routes to various diazenes. A typical one is shown on Scheme. Thus, the addition of monosubstituted hydrazine $2$ to the isocyanate $1$ results in the formation of $1,4$-disubstituted semicarbazide $3$. Oxidation of $3$ to give $4$ is usually performed with either $N$-bromo-succinimide/pyridine or ceric(IV) ammonium nitrate (CAN). The most convenient route to unsymmetrical diazene-di-car-boxa-mide $4c$ is a substitution of the alkoxy group in the diazene $4b$ employing a primary amine as a nucleophile. Diazenes $4b$ as well as their dialkoxy analogs are useful reagents for electrophilic amination of various aromatic compounds. In some cases, the amination process can be accompanied by a halogen migration or by further transformation of an aminated product.

Several diazenes of type $4a-4c$ were found to possess antitumoral activity. Namely, they inhibit the growth of different tumor cell lines. Some of them also reduced the survival of drug-resistant cells, thus offering a new hope for the success in cancer therapy. In addition, most of diazenes are well recognized selective oxidants of various thiols, including natural ones. This characteristic can be crucial in controlling the ratio between monomeric and dimeric glutathione.

Furthermore, diazenedicarboxamides $4c$ are effective inhibitors of D-alanine:D-alanine-ligase (isoform DdlB) from *Escherichia coli*. They also possess anti-bacterial activity, what qualifies them as a promising starting point for further development towards new antibiotics.

A simple method for the preparation of 1,3-di-aryltriazenes $5$ and the synthesis of $N$-acyl-triazenes $6$ as new reagents for $N$-acylation will be presented. It is important to note that compounds $6$ can serve as mild and selective agents for $N$-acylation in various solvents, including protic ones. Very recent results on antitumoral activity of selected 1,3-diaryltriazenes and their $N$-acyl analogues will also be discussed.
FROM DATA TO DRUGS – NOVEL TRENDS IN QSAR

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Chemistry focuses on a construction of a variety of materials of the practical importance and applications, as drugs, preservatives, flavors and so on and the point of the current strategy for the molecular production is a property oriented synthesis. In this context, molecular design is a drug development option that uses fully rational schemes for drug discovery. A drug molecule is a man-made effector designed or discovered in other way to fit the biological counterpart (receptor) and produce the required action. The noise due to the molecular recognition uncertainty in traditional RI m-QSAR cannot be eliminated but by the inclusion of the receptor data. However, a complex nature of ligand-receptor interactions limited the development of the RD m-QSAR. Eventually, with a steady increase of computational power novel RD QSAR methods appeared. This can be divided upon the ligand and receptor representation types applied. Thus, the single receptor and the single ligand data are used in RD 3D-QSAR; the single receptor and the multiple ligand data – in RD 4D-QSAR; the multiple receptor and the multiple ligand data – in RD 5D–QSAR, which is supplemented by multiple solvation data in RD 6D–QAR.

ADMET in silico modeling is expected to be a way towards prediction paradise and the membrane interactions (MI) QSAR approach is a recent RD m-QSAR scheme developed to predict ADMET parameters. Photodynamic therapy (PDT) or surface dyeing are important examples that can profit from this method.

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REFERENCES
PL6
DEVELOPMENT OF NOVEL ANTI-TUMOUR AGENTS BASED ON TARGETING IRON – A CRUCIAL NUTRIENT FOR DNA SYNTHESIS AND PROLIFERATION

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Novel chemotherapeutics with marked and selective anti-tumour activity are essential to develop, particularly those that can overcome resistance to established agents. Iron (Fe) is a crucial nutrient required for cell cycle progression and DNA synthesis and represents a novel molecular target for the design of new anti-cancer drugs. The aim of this current study was to evaluate the anti-tumor activity and Fe chelation efficacy of a new class of Fe chelators, namely the d-2-pyridylketone thiosemicarbazone (DpT) series of ligands.

Our studies using these chelators (Whitnall, M. et al. (2006) PNAS USA 103(20):7670-7675) demonstrated broad anti-tumor activity in 28 different tumor cell lines. Moreover, the compounds could overcome resistance to established anti-tumor agents. The in-vivo efficacy of the most effective chelator identified, Dp44mT, was assessed using a panel of human xenografts in nude mice. These tumours included melanoma, lung carcinoma, ovarian carcinoma and brain (neuroepithelioma). After 7 weeks, net growth of a melanoma xenograft in Dp44mT-treated mice was only 8% of that in mice treated with vehicle. In addition, no differences in these latter animals were found in hematological indices between Dp44mT-treated mice and controls.

No marked systemic Fe-depletion was observed comparing Dp44mT- and vehicle-treated mice, probably due to the very low doses (0.4–0.75 mg/kg/day) required to induce anti-cancer activity. Interestingly, Dp44mT caused up-regulation of the Fe-responsive tumor growth and metastasis suppressor, N-myc downstream regulated gene-1, Ndrg1, in the tumor but not the liver, indicating a potential mechanism of selective anti-cancer activity.

Further studies indicated that the potent anti-tumour activity of the DpT chelators was due to the intracellular formation of a redox-active Fe complex (Richardson, D.R. et al. (2006) J. Med. Chem. 49:6510-6521). Indeed, the high efficacy of these compounds was related to the “double punch“ mechanism. That is, the ability of the chelator to bind Fe which is essential for tumour cell growth followed by the redox cycling of the Fe complex to generate cytotoxic free radicals. Further studies by our group led to the design and synthesis of even more effective agents known as the BpT series of chelators (Kalinowski, D et al. (2007) J. Med. Chem. 50:3716-3729). Again, the efficacy of these latter compounds is related to their ability to form a redox-active Fe complex.

In summary, these results indicate that the novel Fe chelators have potent and broad anti-tumor activity and can overcome resistance to established chemotherapeutics due to their unique mechanism of action.