

# TRENDS IN MEDICINAL CHEMISTRY. HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM, HELD DECEMBER 3, 2015 – NATIONAL HEART & LUNG INSTITUTE, LONDON, UK

R. Davenport<sup>1</sup>, P. Jeffrey<sup>2</sup>, G. Macdonald<sup>3</sup> and R. Porter<sup>4</sup>

<sup>1</sup>Takeda, Cambridge, UK; <sup>2</sup>Rare Disease Research Unit, Pfizer Ltd., UK; <sup>3</sup>Janssen Research & Development, Belgium; <sup>4</sup>Rod Porter Consultancy, UK

## CONTENTS

Summary .....	59
Introduction .....	59
Science, art and drug discovery - a personal perspective .....	59
Changing times and changing paradigms in drug discovery – a Lilly perspective .....	60
Applications of flow chemistry technology in drug discovery and development .....	61
Moving beyond molecular obesity and potency as addictions in drug discovery .....	61
Application of drugability indices within Janssen .....	62
Regulation of stem cells with small molecules .....	63
Natural products and their cellular targets .....	63
References .....	64

## SUMMARY

*Approaches to drug discovery continue to undergo rapid and significant change, presenting both opportunity and challenges. This meeting looked at some of the ways that medicinal chemistry has been responding to these changes organizationally, through the application of new technology and by applying the many lessons learned from past failures. It also focused on the many new opportunities where the application of medicinal chemistry skills could lead to even broader impact in the drug discovery process. This meeting brought together experts to discuss changes in organization that companies have made to respond to changes in the research environment. Application of one emerging technology and its potential to support drug discovery was also discussed. Later sessions examined the roll of the host of metrics for as-*

*sessing compound quality in drug discovery that have emerged and how they may evolve and be used most effectively to drive discovery programs. Finally looking to new areas of opportunity for medicinal chemistry, we heard about the application of medicinal chemistry in stem cell research and the role of chemical biology in the drug discovery process. This meeting was of interest to all involved in the drug discovery process.*

**Key words:** Drug discovery – Flow chemistry technology – Molecular obesity – Drugability indices

## INTRODUCTION

This meeting was dedicated to the memory of Dr. Alma Simmonds, one of the founders of the Society for Drug Research in 1966 which later changed its name to the Society for Medicines Research, who passed away in November 2015. Alma was the first person to be made an honorary life member of the society in the 1980s and she served as Honorary Secretary between 1966 and 1975.

It was Alma together with Norman J. Harper, professor at Aston University, who approached the Pharmaceutical Society in 1966 to let them know about their intention to create this new Society. They were greatly encouraged by the response they got including permission to use the Pharmaceutical Society's Hall for meetings and access to the membership list to publicize the new Society. While the Society has undergone many changes over the years, its focus has remained, since the early days, in providing high-quality scientific meetings to educate and inform the whole of the drug discovery community in academia and industry.

## SCIENCE, ART AND DRUG DISCOVERY - A PERSONAL PERSPECTIVE

*Sir Simon Campbell, CBE FRS, UK*

The meeting was opened by Professor Sir Simon Campbell FRS, former Senior Vice president for Worldwide Drug Discovery at Pfizer. A

.....  
**Correspondence:** secretariat@smr.org.uk.

synthetic organic chemist by training, Professor Campbell received his BSc (1962) and PhD (1965) degrees from Birmingham University in the U.K. Following postdoctoral research positions in the U.K., Chile and U.S., he was appointed visiting professor at the Universidade de Sao Paulo Brasil (1970) before joining Pfizer Central Research in Sandwich, U.K. (1970). Sir Simon's lecture "Science Art and Drug Discovery – A Personal Perspective" began by highlighting the continually changing landscape of the pharmaceutical industry which is under ever increasing pressure to improve productivity and reduce R&D costs. The explosion in genome sciences and associated new technologies offers new opportunities to produce clinically effective and innovative therapies while reducing timelines. However, although new target and hit identification are on the increase, it is the core innovative science of applied drug discovery that continues to be rate limiting in developing high-quality drug candidates capable of going the distance to deliver medicines of true value. Using the discovery and development of the calcium antagonist amlodipine (Norvasc™) and phosphodiesterase 5 (PDE5) inhibitor sildenafil (Viagra™), Professor Campbell illustrated that despite significant gaps in receptor and enzyme structure, the research programs were ultimately successful through the correct blend of scientific data, experience, intuition and an appropriate level of controlled and measured risk taking. The objective of the amlodipine program was to develop a once-daily calcium antagonist for the treatment of angina and hypertension that was a significant improvement over the current standard of care, nifedipine (t.i.d. dosing), diltiazem (significant CYP-mediated drug–drug interaction liability) and verapamil (narrow therapeutic index). The poor pharmacokinetic profile common to these agents offered the opportunity for significant improvement but at the time there were over 90 published patents around the parent dihydropyridine ring system that posed a significant challenge in establishing a strong proprietary position. An advocate of Pasteur's Dictum "chance favors the prepared mind," Professor Campbell and his team employed a rational medicinal chemistry strategy focused on improved metabolic stability which delivered a novel series of dihydropyridines with high oral bioavailability and associated plasma terminal half-lives suitable for once-a-day dosing. Key to the selection of UK-48,340 was a revision of the in vivo preclinical protocol to understand the time course of the pharmacological effect rather than look at effects at a single time point. Following extensive pharmacological profiling, UK-48,340 (UK-48340-26, amlodipine besylate) was selected for clinical development and was subsequently approved as Norvasc. Therapeutic dose is low (5–10 mg q.d.) and steady-state plasma concentrations are achieved after about 5 days due to the long plasma terminal half-life (30–50 hours) which was attributed to a significantly increased volume of distribution. Compared with other dihydropyridines, UK-48,340 potentiated a hydrophilic interaction and formation of a salt bridge within the lipid membrane leading to favorable disposition properties. The low dose and long terminal half-life coupled with low drug accumulation means that patients treated with Norvasc can tolerate accidental "drug holidays" leading to an overall improvement in patient compliance. To date over 30 billion patient days of therapy have been achieved since launch.

Nitric oxide (NO) diffuses into vascular smooth muscle cells, stimulating the production of cGMP leading to vasodilation. The effects of nitric oxide and cGMP are limited by PDE5 which inactivates cGMP in the smooth muscle of the vascular and in platelets. Starting with za-

prinast, a weak, nonselective PDE5 inhibitor, computer modeling techniques guided medicinal chemistry design efforts to achieve a significant increase in potency and selectivity with a novel series of pyrazolopyrimidines that were identified as highly potent PDE5 inhibitors with potential utility in the treatment of angina pectoris. UK-92,480 (sildenafil) was demonstrated to have very good potency and excellent selectivity over PDEs 1–4. However, while early clinical trials with UK-92,480 were unsuccessful, experimental and clinical studies provided evidence that PDE5 inhibition might be an attractive therapeutic approach to erectile dysfunction, as NO is a key regulator of vascular tone in the corpus cavernosum. By 1997, 21 separate clinical trials in over 5,000 patients had demonstrated the efficacy of sildenafil in various patient populations. The FDA approved Viagra in March 1998 with European approval following in September 1998. One of the most widely prescribed medicines, over 120 million prescriptions by some 600,000 physicians for over 20 million men worldwide, Viagra continues to be evaluated in additional indications including pulmonary hypertension associated with underlying lung diseases, chronic thromboembolic pulmonary hypertension, Raynaud's phenomenon, right- and left-ventricular hypertrophy and cerebrovascular diseases. Key to both of these highly successful research programs were a number of guiding principles advocated by Professor Campbell: be hypothesis driven and generate the answers to the key questions; develop robust, clinically focused biology screening cascades with appropriate stage gates and progression criteria; utilize state-of-the-art platforms but, perhaps most importantly, recognize from the outset that although drug discovery requires a multidisciplinary and integrated approach, too large a team or unit can be ineffective. In concluding, Professor Campbell mused that perhaps the concept of Dunbar's Number of 150 people is as equally important in drug discovery as it is in maintaining social relationships.

## CHANGING TIMES AND CHANGING PARADIGMS IN DRUG DISCOVERY – A LILLY PERSPECTIVE

*Dr. Magnus Walter, Lilly, UK*

Presenting "Changing Times and Changing Paradigms in Drug Discovery – a Lilly Perspective", Dr. Magnus Walter reflected on the recent period of extensive change the pharmaceutical industry has experienced, and continues to do so. Efforts to improve productivity and reduce costs and attrition rates have resulted in the need to radically change the drug research business model. While the maxim of "hypothesis – design-prepare-test" is still at the core of medicinal chemistry thinking, new technologies, synthetic methods and increased automation require different more cost-effective approaches. Consequently, today's medicinal chemistry is no longer a totally internal-based resource but a hybrid mix of both internal and external efforts. In order to reduce fixed overhead costs, improve flexibility and access new technologies and scientific talent during the period 2002 to 2010, Lilly externalized medicinal chemistry support across all stages of the portfolio using multiple partners across the globe with the result that by 2010, > 50% of new compound registrations were attributed to external efforts. However this was not without risk. The economic benefits became less attractive as competition increased and capacity decreased, especially in the Asian sector. Local infrastructure did not always keep pace with the requirements for rapid, iterative drug discovery and externalization still required a significant operational overhead. Describing the next iteration as "Insourcing", Dr. Walter

highlighted the key benefits of co-location and maximizing the use of existing laboratory facilities within the parent company. This approach reduced both the operational infrastructure associated with external partnering and the burden on Lilly scientists managing third-party interactions and improved compound iteration and cycle times. Originally trialed in 2012 at the Indianapolis site, this model is now a global partnership utilizing a total of 35 FTEs across the U.S. and U.K. Lilly sites. Lilly's Open Innovation Drug Discovery (OIDD) program ([www.openinnovation.lill.com](http://www.openinnovation.lill.com)) is founded on the principals that pharmaceutical companies, academic and public research institutions all share a common desire to advance innovative biomedical science and research. This collaborative platform unites external investors with Lilly scientists providing expertise for external investigators. The OIDD web-based interface protects participant contributions and all data generated is owned by the submitting investigator or institution. OIDD also offers the capability of participants to request compound synthesis and subsequent additional in vitro profiling and all proposed compounds remain structure blinded through the conclusion of the proposed selection process. Since its formation, over 600 scientists from over 300 affiliations in 25 counties have participated in this collaborative venture, over 360,000 structures have been uploaded and 22 research collaborations have been initiated. In concluding, Dr. Walter commented that while the old days of "know-it-all and own-it all" pharmaceutical companies are very much in the past, there are still a number of challenges remaining. Increasing compound numbers alone does not improve drug discovery effectiveness, good design and judgement are just as important, if not more so, than scalable and effective synthetic processes. Thoughtful design and discovery aligned with a fundamental understanding of target engagement related to pharmacological response are key drivers for success. In these aspects medicinal chemistry is still very much a central discipline in successful drug discovery.

## APPLICATIONS OF FLOW CHEMISTRY TECHNOLOGY IN DRUG DISCOVERY AND DEVELOPMENT

Dr. Chris Selway, Cyclofluidics, UK

The technique of flow chemistry has developed rapidly over the last 15 years, and is now being applied routinely by academic and industrial research organizations. A number of commercial systems exist, with flow rates ranging from as low as 10  $\mu\text{L}/\text{min}$  to 20  $\text{mL}/\text{min}$ , and the ability to control mixing rate, heat transfer (heating and cooling), as well as multistep chemistries makes it a very versatile technique. Flow chemistry has also enabled access to types of chemistry (such as photochemistry, electrochemistry and surface chemistry) which have been difficult using standard batch chemistry. Cyclofluidic (a private/public funded initiative) was setup to design an Integrated Drug Discovery System, which would integrate flow chemistry, purification, analysis and biology screening for rapid hit to lead optimization. Combining this further with integrated design software, molecules can be synthesized and screened in a fully automated serial iterative mode, using the biological data generated on each cycle to inform the selection of the best compound to make next. This platform has been named CycLOps™ and has reduced the synthesis to screen cycle time to 90 minutes in a proof-of-concept study (Fig. 1). Within 142 synthesis and screening cycles, a 22 nM, 6000-fold selective lead was generated from a 1  $\mu\text{M}$ , 30-fold selective hit.

## MOVING BEYOND MOLECULAR OBESITY AND POTENCY AS ADDICTIONS IN DRUG DISCOVERY

Dr. Mike Hann, GlaxoSmithKline, UK

Dr. Hann reflected on some of the learnings about causes of attrition in drug discovery over recent years and how medicinal chemistry practice can contribute to alleviating these issues. Some solutions were

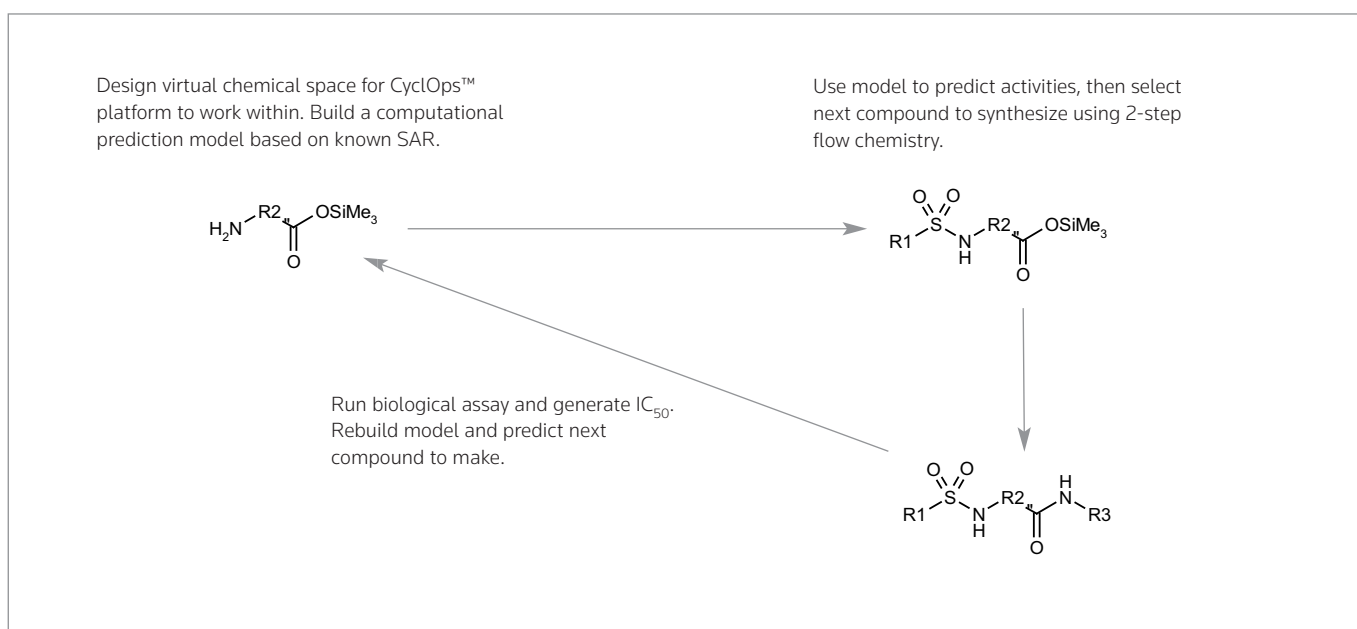


Figure 1. CycLOps™ proof of concept study.

also offered, including discussion of the work at GlaxoSmithKline (GSK) on determining intracellular drug concentrations which historically has been a stumbling block for many intracellular targets. Molecular obesity (1)—the tendency of molecules to become too large and particularly too lipophilic for their own good in a drive for potency—is now well recognized as presenting a substantial risk of attrition downstream. To manage molecular obesity, various indices have now been introduced including ligand efficiency and lipophilic ligand efficiency. While potency (usually determination of which is the first and most rapidly generated piece of data for a new compound) can be beneficial in improving therapeutic index, selectivity and reducing drug dose, it is too often achieved by increasing both molecular weight and lipophilicity. As secondary assays may well be cellular, the tendency to strive for early cellular activity is to increase lipophilicity providing an additional driver into inappropriate physicochemical space. Furthermore, as Dr. Hann clearly demonstrated, increasing lipophilicity drives towards reduced target selectivity (2). Potency as determined by the Gibbs free energy ( $= -RT\ln K_d$ ) is determined by both enthalpy—largely driven by polar interactions and entropy broadly associated with lipophilic interactions. More potent compounds have been shown to have a much larger entropic contribution relative to less potent compounds with a switch to greater entropic relative to enthalpic input at pK<sub>d</sub>s beyond 8. Thus, implicit in the drive to very potent compounds is increased lipophilicity. The message from Dr. Hann was to ensure getting the most from polar enthalpic interactions at an early stage in lead optimization was perhaps more readily done with fragments as starting points (3, 4). Historically, potency of marketed compounds—where their target has been clearly identified, clusters around a  $-\log_{10}$  affinity 7.8—in other words potency despite being (usually) easy to measure is not a great indicator of ultimate success for a compound (5). The Property Forecast Index ( $PFI = mChromLogD_{7.4} + \# \text{ aromatic rings}$  [6]) has been found by the GSK group to be a useful guide to developable compounds with lower values, up to 6, improving solubility, reducing plasma protein binding, and improving metabolic stability and reduced P450 interactions. However, optimal permeability in an artificial membrane assay is between PFI 6–8; further more potency tends to increase with increasing PFI. Thus, some compromise is invariably required although the PAMPA permeability measurements are a rate not an equilibrium parameter. Solubility is of course a key component in getting compounds to the site of action—the first of the Pfizer three/four pillars concept (7, 8). One perspective on the “first pillar” is via a drug efficiency index ( $\text{Drug Efficiency Index} = \text{Log}[\text{Biophase Concentration} \times 100/\text{Dose}] + pXC_{50}$ ) (9), where high drug efficiency needs a balance of potency and access to the target and equates to a low dose. Remarkably there is a reasonable correlation between an in vitro drug efficiency measure reliant on two HPLC methods ( $\text{In vitro HPLC log Deff max} = 2 - (0.23 \times \text{logk HSA} + 0.43 \times \text{logkIAM} - 0.72)$ ) (10) and in vivo drug efficiency. Continuing the theme of “Pillar one” - while biophase concentration is generally taken to refer to plasma concentration, this is increasingly an oversimplification with many new targets now being intracellular. In a final section of his presentation, Dr. Hann discussed the application of RapidFire MS to determine total cellular drug concentrations; results are expressed as a ratio of intracellular to extracellular concentration (11). Interestingly, there was little correlation of results with artificial membrane permeability although there was some with  $\text{log}D_{7.4}$  and even more with artificial membrane affinity. In a next step, intracellular free concentration was estimated using

Rapid Equilibrium Dialysis. Analysis of results indicated that lower PFI was generally indicative of higher intracellular free drug and free fraction than compounds with higher PFI, although too low a PFI will not allow compounds to pass through the membranes in the first place. Following the “Pillar one” theme of “Does it get there?” was a short discussion of the development of Secondary Ion MS (SIMS) which was demonstrated to give subcellular resolution of drug from in vivo samples. This technique clearly has a lot of promise for future understanding in this question of cellular disposition. For the second of the Pfizer Pillars—Does the compound engage the target?—Cellular Thermal shift assays were seen as a new approach. The third of the Pfizer pillars—Does the compound elicit the desired response?—is dependent on both the choice of target and chemical tractability and Dr. Hann emphasized both target and chemistry need to be tractable for a program to be tractable. Finally Dr. Hann highlighted the importance of remembering historical learnings, the “unknown knowns”, not least of which was the emphasis on maintaining a reasonable logP discussed by Hansch in 1969. Perhaps an alternative expression of this is how can we “stand on the shoulders of giants” if we forget, ignore or are unaware of the giants!

#### APPLICATION OF DRUGABILITY INDICES WITHIN JANSSEN

*Dr. Gary Tresadern, Janssen, Belgium*

Dr. Gary Tresadern, talked about the application of various drugability indices within CNS projects at Janssen in his talk entitled “Application and Analysis of Drugability Indices at Janssen”. After reviewing the various parameters commonly used in compound optimization (logP, LE [ligand efficiency], LLE [lipophilic ligand efficiency], LELP [lipophilicity-corrected ligand efficiency], etc.) and how these differentiate drugs, leads and hits, Dr. Tresadern presented several hit and lead optimization programs from the Janssen Neuroscience team and how such parameters had evolved over the lifetime of the project. A particular focus was placed on the optimization of allosteric modulators (mGlu<sub>2</sub>, mGlu<sub>5</sub> and  $\gamma$ -secretase), where it is known that potent compounds often reside within an area of chemical space that is characterized by high lipophilicity, high molecular weight and low solubility. Traditionally, optimization programs aimed at increasing compound potency have done so at the expense of increasing lipophilicity, which may in part be related to the nature of the binding site. In analyzing the progress of compound optimization from HTS hit to preclinical candidates in mGluR modulator programs, project teams appeared to generally start from hits with good LE (low molecular weight), but with poor LLE (moderate to high logP and low potency). As hits were subsequently optimized following good medicinal chemistry design, LLEs were significantly improved, with potency being increased and logP being kept constant and under control. Ultimately, preclinical candidates were shown to reside in a more optimal area of chemical space, characterized by low molecular weight, moderate lipophilicity and good LLE. It was noted that by maintaining a tight control over lipophilicity, compounds of high potency and high LLE could be designed within the same series. An additional conclusion was that selecting hits based on LE and LLE moved the program into potential more attractive chemical space during subsequent optimization stages. Finally, a comparison was made with compound optimization for two different mechanisms (PDE10 inhibition and D<sub>2</sub> antagonism), where achieving high LE and LLE was much more achievable, likely due to the target classes concerned.

The modulation of  $\gamma$ -secretase was highlighted as a particularly challenging mechanism to be able to design potent compounds that maintain acceptable molecular weight and lipophilicity, with reported modulators often residing at the limit of 'CNS drugable space'. Hit and lead compounds generally had poor LLE, with this parameter not changing significantly over the course of the optimization program. Late stage leads generally had higher LLE values, but still much lower than in other programs, due largely to the inherent higher lipophilicity. Compounds with these moderate LLEs were subsequently shown to suffer from problems such as poor pharmacokinetics and liver toxicity. Emerging from a subsequent back-up program looking for significantly different chemistry, new leads were identified that appeared to offer an improvement in LLE values in the 4.5-5.0 range.

In conclusion, for targets where potency could often be increased at the cost of increased lipophilicity, driving optimization on LLE was shown to provide late-stage compounds of much higher quality. It was shown that while selecting hit compounds based on LE was beneficial (favoring low molecular weight starting points), LLE was a much more useful metric for the subsequent optimization phase. Finally, the nature of the target class and mode of action of compounds had a significant effect on how effectively drugability indices such as these could be used.

## REGULATION OF STEM CELLS WITH SMALL MOLECULES

Dr. Yen Choo, Progenitor Therapeutics, UK

Being able to differentiate human embryonic stem cells or iPSC into different cell types offers the chance to access therapeutically relevant cell therapies, but also access drug discovery programs to identify disease-modifying small-molecule drugs. Small molecules are recognized as being cheaper, more stable and reliable than the recombinant growth factors currently used to drive differentiation. CombiCult™ (a Plasticell technology) is a high-throughput screening system capable of multiplexing extremely large numbers of putative

cell differentiation protocols to rapidly pinpoint combinations of variables that drive stem cell differentiation into given phenotypes. This is achieved by stem cells being grown on microscopic beads which are shuffled serially through many different culture conditions, which are tracked by unique fluorescent tags and de-convoluted using proprietary bioinformatics software. The best yielding conditions can then be independently optimized and scaled up to yield the desired functional cell population. Progenitor Therapeutics Ltd. has used CombiCult to differentiate pluripotent stem cells into myeloid progenitor cells, which when screened phenotypically for the appearance of megakaryocytes and platelets identified Promacta (eltrombopag, GSK) a marketed small-molecule TPO mimetic. CombiCult was later applied to generate oligodendrocyte progenitor cells in 15 days, yielding 80% pure OPCs (a significant improvement over the 98-day published protocol). These were screened against libraries of bioactive compounds, which identified small molecules which aid remyelination of neurons, as a potential treatment of multiple sclerosis. Further cells have also been generated, screened and molecules that stimulate the regeneration of specific cells, restoring tissue function in serious degenerative diseases such as osteoarthritis and muscular dystrophy identified.

## NATURAL PRODUCTS AND THEIR CELLULAR TARGETS

Prof. Stephan Sieber, Munich, Germany

Prof. Sieber discussed some of his latest work on the use of electrophilic natural products to help in identifying new targets through proteomics with the possible progression to new therapeutic approaches. Focus was on the search for unprecedented antibiotic targets moving away from mechanisms addressing cell wall biosynthesis, protein biosynthesis or RNA/DNA replication and where antibiotic resistance routinely appears. Also discussed by Prof. Sieber were approaches to oncology targets. There are a substantial number of naturally occurring electrophilic  $\beta$ -lactones, some of which are re-

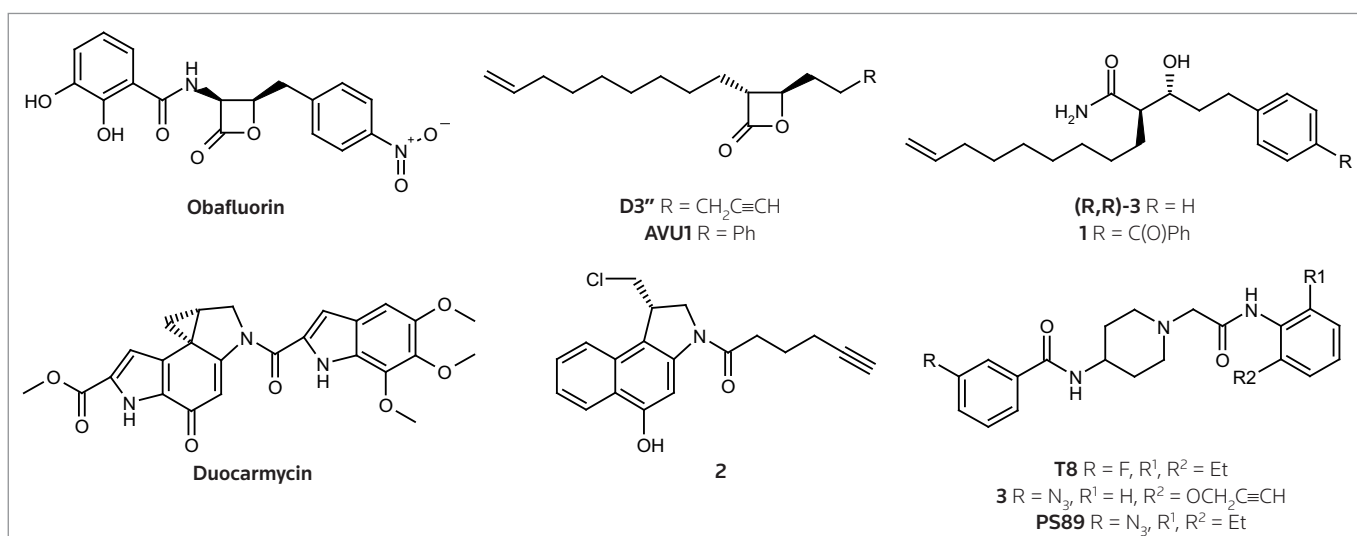


Figure 2. Structures for discussed compounds.

ported to have weak antibiotic activity including obaflourin (Fig. 2). To explore molecular targets for these lactones, a small library of alkynyl substituted analogues was prepared. Incubation with the target organisms proteome and subsequent tagging with an azide linked biotin tag and fishing with avidin. Proteins labeled by covalent ring opening of the  $\beta$ -lactone were thereby isolated and could be analyzed following SDS-gel analysis, tryptic digest and LC-MS/MS. An example of this work was the discovery that compound D3 labeled caseinolytic protease P (ClpP) in *S. aureus*. ClpP serves to remove misfolded proteins as a result of a stress response and is associated with virulence response in bacteria (13). ClpP readily accommodates the  $\beta$ -lactone as a substrate (14, 15) which acts as an irreversible inhibitor reducing the generation of mediators of inflammation, sepsis and toxic shock. While compound AVU1 was active in a topical mouse model of *S. aureus*-induced abscess, progression of  $\beta$ -lactones is severely limited by their instability in plasma. The amide (*R,R*)-3 corresponding to the amide of ring opened AVU1 showed good plasma stability but also antivirulence activity against a range of clinical isolates and with no evidence for resistance. However the activity of (*R,R*)-3 was not associated with inhibition of ClpP. To determine the target of (*R,R*)-3, a photo labile benzophenone cross linker was attached to (*R,R*)-3 along with an alkyne to exploit the previously used click chemistry to introduce a biotin tag—compound **1**. Intriguingly, **1** was identified as binding to a manganese transporter protein knockout of which was shown to reduce bacterial virulence. Importantly, (*R,R*)-3 showed substantially improved pharmacokinetics relative to  $\beta$ -lactones and was active on systemic treatment in the mouse *S. aureus* abscess model. Prof. Sieber moved on to describe two aspects of his work in identifying targets for cancer therapies. Firstly in a collaboration with Prof. Tietze was work to identify targets of duocarmycin. While DNA is the prominent target for duocarmycins, Prof. Siebers work showed that appropriately tagged duocarmycin analogues also targeted aldehyde dehydrogenase 1A1 (ALDH1A1) which is essential for lung cancer cell survival and is also involved in detoxification of chemotherapeutics. Furthermore, removing the indole motif prevented interaction with double stranded DNA while retaining ALDH1A1 inhibitory activity, e.g., **2**. Compounds showed excellent selectivity for ALDH1A1 relative to other aldehyde dehydrogenases binding covalently with Cys302. Compounds such as **2** retained excellent cytotoxicity attributed to their ALDH1A1 inhibitory activity and consistent with siRNA knockout experiments. Finally, Prof. Sieber discussed his work in collaboration with Prof. Vollmar to identify the target of compound T8, a sensitizer of cancer cells towards cancer therapeutics such as etoposide. A photolabel and alkyne tag were introduced to T8 to give compound **3** which on incubation with cells, photolabeling in situ, cell lysis and subsequent azide/biotin tagging allowed extraction and identification of labeled proteins. Targets for this compound were identified as several protein disulphide isomerases (PDI) which support cancer cells when they are under stress. From testing of a range of analogues, the azide PS89, a reversible inhibitor, was the most efficacious at cell sensitization in the presence of etoposide with the azide binding deep into a sub-pocket. PS89 is superior to previous poorly selective or irreversible inhibitors and will be a valu-

able tool for the studies of the role of PDI in cancer.

## DISCLOSURES

R. Davenport is an employee of Takeda. P. Jeffrey is an employee of Pfizer. G. Macdonald is an employee of Janssen (pharmaceutical companies of Johnson & Johnson). R. Porter states no conflicts of interest.

## REFERENCES

- Hann, M.M., *Molecular obesity, potency and other addictions in drug discovery*. MedChemComm 2011, 2(5): 349-55.
- Leach A.R., Hann, M.M. *Molecular complexity and fragment based drug discovery ten years on*. Curr Opin Chem Biol 2011, 15(4): 489-96.
- Ferencyz, G.G., Keseru, G.M. *Enthalpic efficiency of ligand binding*. J Chem Inf Model 2010, 50(9): 1536-41.
- Hann, M.M., Keseru, G.M. *The challenge of medicinal chemistry – the role for nature and nurture in lead discovery and optimization*. Nat Rev Drug Discov, in press.
- Overington, J.P., Al-Lazikani, B., Hopkins, A.L., *How many drug targets are there?* Nat Rev Drug Discov 2006, 5(12): 993-6.
- Young, R.J., Green, D.V.S., Luscombe, C.N., Hill, A.P. *Getting physical in drug discovery II. The impact of chromatographic hydrophobicity measurements and aromaticity* Drug Discov Today 2011, 16 (17/18): 822-30.
- Morgan, P., Van Der Graaf, P.H., Arrosmith, J. et al. *Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving phase II survival*. Drug Discov Today 2012, 17(9/10): 419-24.
- Bunnage, M.E., Piatnitski Chekler, E.L., Jones, L.H. *Target validation using chemical probes* Nat Chem Biol 2013, 9(4): 195-9.
- Braggio, S., Montanari, D., Rossi, T., Ratti, E. *Drug efficiency: a new concept to guide lead optimization programs towards the selection of better clinical candidates*. Exp Opin Drug Discov 2010, 5(7): 609-18.
- Valko, K.L., Nunhuck, S.B., Hill, A.P. *Estimating unbound volume of distribution by in vitro HPLC-based human serum albumin and immobilized artificial membrane-binding measurements*. J Pharm Sci 2011, 100(3): 849-62.
- Gordon, L.J., Allen, M., Artursson, P. et al *Direct measurement of intracellular compound concentration by RapidFire mass spectrometry offers insights into cell permeability*. J Biomol Screen 2016, 21(2): 156-64.
- Mateus, A., Matsson, P., Artursson, P. *A high-throughput cell-based method to predict the unbound drug fraction in the brain*. J Med Chem 2014, 57(7): 3005-10.
- Staub, I., Sieber, S.A., *-Lactam probes as selective chemical-proteomic tools for the identification and functional characterization of resistance associated enzymes in MRSA*. J Am Chem Soc 2009, 131(17): 6271-6.
- Zeiler, E., List, A., Alte, F. et al. *Structural and functional insights into caseinolytic proteases reveal an unprecedented regulation principle of their catalytic triad*. Proc Nat Acad Sci U S A 2013, 110(28): 11302-7.
- Gersch, M., List, A., Groll, M. et al. *Insights into structural network responsible for oligomerization and activity of bacterial virulence regulator caseinolytic protease P (ClpP) protein*. J Biol Chem 2012, 287(12): 9484-94.