

# FROM TARGETS TO CANDIDATES: EMERGING STRATEGIES IN DRUG DISCOVERY

## HIGHLIGHTS FROM THE SOCIETY OF MEDICINES RESEARCH SYMPOSIUM, HELD ON DECEMBER 12<sup>TH</sup>, 2013 – NATIONAL HEART & LUNG INSTITUTE

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### CONTENTS

Summary .....	147
Fragment-based drug discovery for allosteric sites, PPI's... and the future .....	147
Targeting protein-protein interactions using small-molecule fragments and an antibody platform .....	149
Antibodies as tools in NCE discovery .....	150
Challenges and opportunities in allosteric enzyme inhibition .....	150
Phenotypic screening approaches for the discovery of novel antimalarials .....	151
Bicycles: high affinity bicyclic peptides from phage display libraries .....	153
Leading into new spaces – strategies for lead generation in neuroscience medicinal chemistry .....	154
References .....	155

### SUMMARY

*New drug discovery strategies, facilitated by technological advances, have evolved rapidly in recent years. Drug researchers are now pushing the drug discovery envelope, particularly in the fields of hit identification, prosecution of new targets and exploration of new chemical space. This symposium brought together experts to highlight several of these developments, describing work from hit identification through to candidate selection. The themes covered included: application of fragment-based drug discovery to allosteric sites and protein-protein interactions; the use of antibodies to inform new small-molecule*

*design; phenotypic screening approaches for the discovery of novel antimalarials; challenges and opportunities in allosteric enzyme inhibition; and the use of bicyclic peptides and macrocycles to open up new drug discovery avenues.*

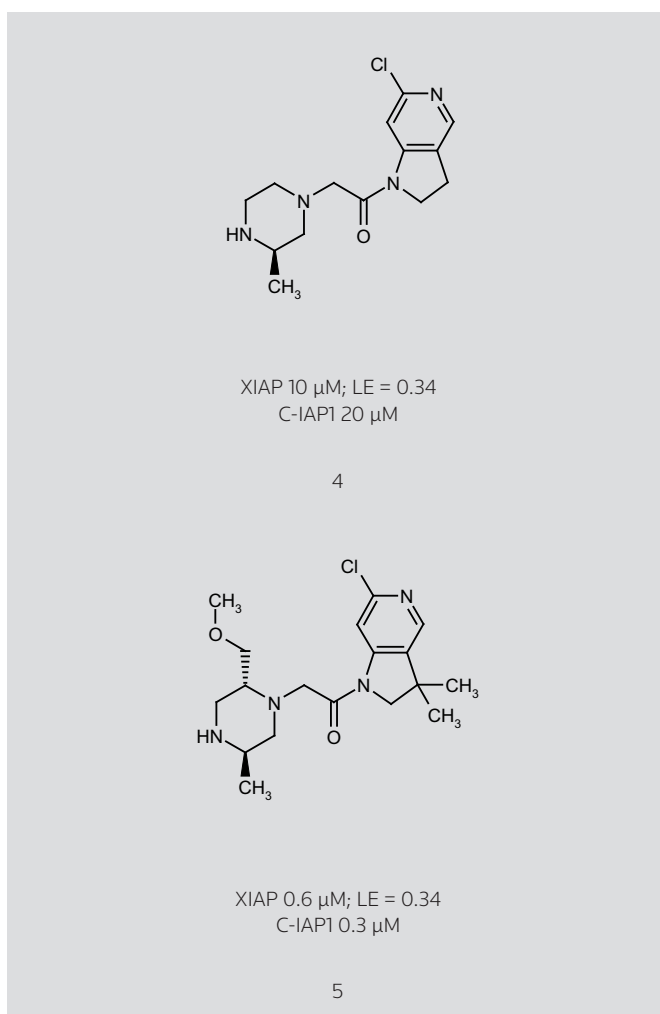
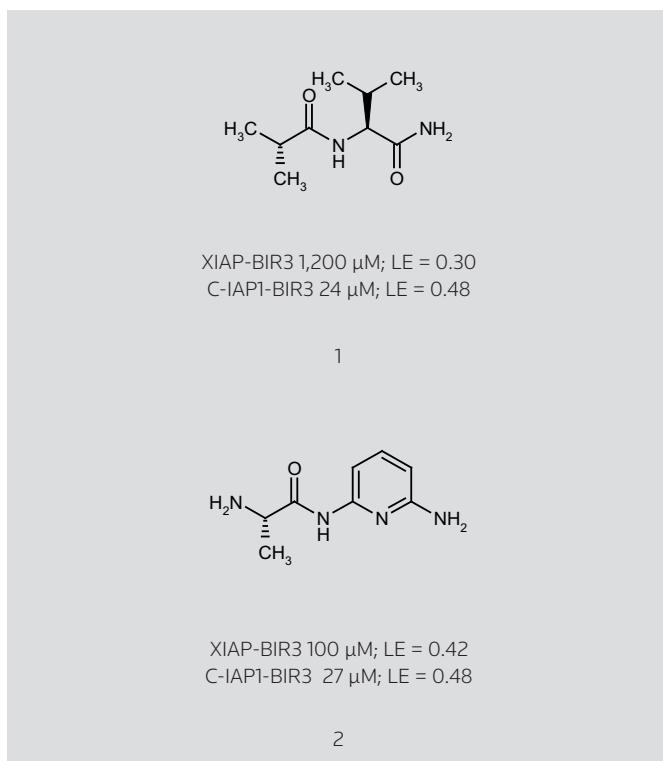
**Key words:** Drug discovery strategies – Targets – Allosteric sites – Antibodies – Antimalarials – Bicyclic peptides

### FRAGMENT-BASED DRUG DISCOVERY FOR ALLOSTERIC SITES, PPI'S... AND THE FUTURE

Dr. David Rees (Astex Pharmaceuticals, U.K.) discussed the application of the AstexPyramid™ fragment-based drug discovery (FBDD) platform to discover inhibitors of protein-protein interactions (PPIs). Inhibitor of apoptosis proteins (IAPs) are a family of proteins with a high degree of conserved structure and function that serve to promote cancer cell survival by acting as endogenous inhibitors of apoptosis. Structurally, the proteins are characterized by one to three baculovirus IAP repeat (BIR) domains that interact with apoptotic target proteins, such as caspases. Of interest in cancer therapy are antagonists of IAPs, in particular, X-linked IAP (XIAP), which target caspases 3, 7 and 9, and C-IAP1 and C-IAP2, which regulate caspase 8 via TNF- $\alpha$  signalling mechanisms (1). Using structural-based drug design in combination with endogenous antagonists of IAPs, first-generation IAP antagonists have advanced to the clinic. However, the majority are peptidomimetic in nature, with an alanine warhead and selectivity for C-IAP1/2 over XIAP (2). Astex's program focused on developing a non-alanine dual C-IAP1 and XIAP antagonist. One of the challenges surmounted was obtaining robust soakable crystal forms. Four BIR domains were successfully crystallized (XIAP-BIR3, C-IAP1-BIR3, C-IAP2-BIR3 and ML-IAP) and used to acquire structural information. A further demand centered on the need to fragment screen at a high concentration (0.1 M) using long soaking times (48 hours). As a consequence, only fragments with high built-in solubility proved amenable to these screening conditions. Using the XIAP-BIR3 crystal structure in tandem with 1D and

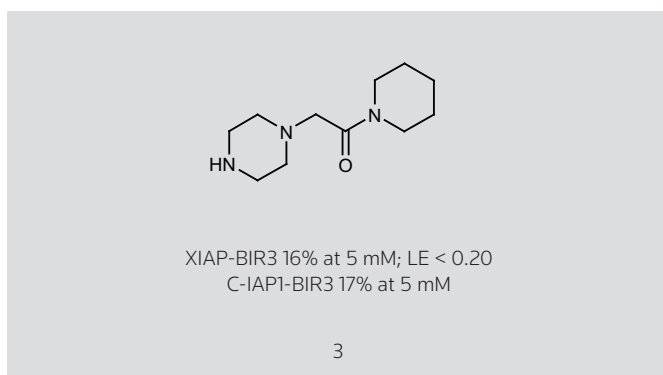
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2D NMR experiments, screening of 1,200 fragments delivered potent alanine hits with good ligand efficiency (LE) and selectivity for C-IAP1 over XIAP, as exemplified by **1** and **2**.



Of more interest was the identification of the non-alanine hit **3**, which appeared to be a very weak binder of both XIAP and C-IAP1.

Further optimization of **3** led to fragment **4** and early lead **5**, which showed a balanced C-IAP1/XIAP profile, with  $> 10^4$ -fold improvement in affinity. X-ray crystal structures of alanine fragment **1** and optimized piperazine fragment **4** in XIAP-BIR3, overlaid with key



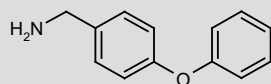
amino acid residues from the C-IAP1-BIR3 protein crystal structure, revealed the alanine warhead formed charge–charge interactions with two acidic residues in C-IAP1, but only one in XIAP, thus accounting for its greater affinity toward C-IAP1. In contrast the balanced activity of the piperazine warhead resulted from charge–charge interactions with one acidic residue in both C-IAP1 and XIAP, together with H bonds to conserved backbone amino acids.

Lead compound **5** delivered through a further cycle of compounds the clinical candidate AT-IAP (structure undisclosed), which showed XIAP-BIR3 of  $< 0.04 \mu\text{M}$  and C-IAP1-BIR3 of  $< 0.01 \mu\text{M}$ , with associated potent cellular activity for XIAP cells of 5.1 nM and C-IAP1 cells of 0.3 nM. Dose-dependent inhibition of tumor size was demonstrated in breast and melanoma mouse xenograft models (3).

The power of fragment screening was equally demonstrated in the discovery of an allosteric site on the hepatitis C virus (HCV) helicase protease, leading to new thinking in the regulation of HCV serine protease NS3 protein function and potential development of a new

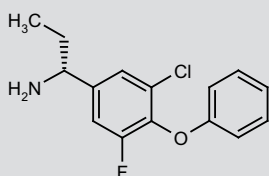
class of antiviral agents (4). The use of soakable crystals of HCV serine protease NS3/non-structural protein 4A (NS4A) full-length genotype 1b holoenzyme was critical to successful detection of bound fragments in a novel site located at the interface between the protease and helicase domains. Using structural guided optimization, the initial starting fragment hit **6** was tuned to **7** and lead compound **8**.

Lead compound **8** demonstrated no activity against the isolated protease domain, yet it elicited submicromolar activity in an HCV replicon assay, with no cytotoxicity, supporting the concept that the allosteric site identified was only functionally active in the full-length protein. This was further corroborated from mutant generation, where viral resistance associated with **8** mapped to V630L or M485V located on the allosteric site where **8** bound. Based on the available information, a mode of action of the allosteric inhibitors was proposed in which the protease–helicase interface is stabilized in a closed autoinhibited conformation. In concluding remarks, it was



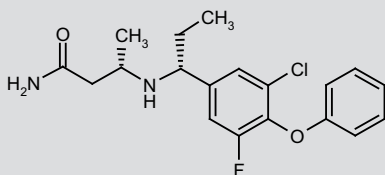
≈ 500 μM (LE = 0.30)

**6**



2.1 μM (LE = 0.41)

**7**



0.11 μM (LE = 0.38)

**8**

suggested that fragment-based methods were well suited to PPIs and allosteric targets, but challenges existed in obtaining soakable crystal forms and optimizing mM fragments into nM leads. The Astex FBDD process continues to evolve to meet these demands, with a seventh generation library now in use containing fragments of high solubility, which are often a prerequisite with such targets.

### TARGETING PROTEIN–PROTEIN INTERACTIONS USING SMALL-MOLECULE FRAGMENTS AND AN ANTIBODY PLATFORM

Dr. Rich Taylor (UCB, U.K.) described UCB's approach to FBDD targeting PPIs. Over a number of years, the UCB fragment collection has evolved from the initial throws of creating a pool of fragments from commercial sources, approximately 2,500 in number, selected and filtered using simple 1D and 2D properties (so called G1), to one incorporating additional proprietary scaffolds from internal projects (so called G2) to the current day, where a fragment collection exists that has been carefully designed to cover all commercial fragment space (so called G3). The path to G3 resulted from prosecuting commercial compounds with rule-of-three compliance to identify 360K fragments, which was reduced to 70K fragments after filtering reliable suppliers, removal of reactive groups and non-drug like functionality, and the use of Stardrop solubility calculations. The key to reducing this further to a manageable number lay in the use of ring-based clustering techniques, from which 30K fragments were identified and 23K added to the screening deck after quality control. To address how well the fragment collection G3 covered drug-like space, the database of oral new chemical entity (NCE) drugs from the FDA orange book was computationally divided, using recursive algorithms into ring fragments and ring systems, and its distribution compared to the G3 fragment deck. Results suggested that 70% of non-singleton drug ring systems were captured in G3. Further analysis of the FDA orange book revealed some interesting facts, namely that over a 28-year period, on average 28% of new drugs each year contained one novel ring system that has not been previously used in an approved drug, and furthermore, only 0.6% of new drugs contained more than one new ring system.

Biacore technology was utilized to screen such a large collection of fragments, with a set-up that allowed screening of 20,000 fragments against 12 targets in one month using three machines. The advantages of biacore technology lay in low protein consumption, coupled with high sensitivity, which allowed the detection of fragment binding in the mM range. Further information regarding binding kinetics ( $k_{on}$ ,  $k_{off}$ , shape of the curve) proved useful in the prioritization of fragment hits. Immobilization of the protein to the sensor chip surface usually entailed amine coupling. In particular, the use of a lysine tag (both N and C forms) gave increased specificity while maintaining capacity. Although biacore screening represented the work horse, other additional techniques were utilized where necessary to confirm hits or provide additional binding information, such as fluorescence resonance energy transfer (FRET), NMR (HSQC and STD), X-ray crystallography, mass spectrometry and isothermal titration calorimetry (ITC).

The G3 fragment collection was screened against 12 PPI targets simultaneously, which based on the literature had never been prosecuted with a fragment library. Binding hit rates reached approxi-

mately 1%, with 964 unique hits hitting any 12 targets and 908 hitting  $\leq 8$  targets. One of the PPI targets was the cytokine interleukin-6 (IL-6), which when unregulated has been implicated in the pathogenesis of a variety of diseases, including rheumatoid arthritis (RA), Crohn's disease and systemic lupus erythematosus (SLE). IL-6 is known to interact with receptor gp80 on the surface of immune cells. A co-receptor, gp130, is then recruited to form a complex which signals into the cell via the JAK-STAT signaling pathway. Olokizumab, a UCB anti IL-6 monoclonal antibody (MAb) blocks the IL-6::gp80 + gp130 trimer formation and has delivered clinical efficacy in RA patients. From biacore screening, multiple fragments were identified, with  $K_d$ 's ranging from approximately 50  $\mu\text{M}$  to  $> 1$  mM. Through a collaboration with Proteros, several fragments were successfully crystallized with the IL-6::gp80 complex and the structures were solved. The small-molecule ligands bind between IL-6 and gp80 and require both proteins to be present. Of greater interest is that binding is away from where the IL-6::gp80 dimer binds to gp130, such that binding could be considered allosteric in nature. The protein motion caused by the small molecule ligands mirror that observed for binding of the antibody olokizumab.

#### ANTIBODIES AS TOOLS IN NCE DISCOVERY

Dr. Alastair Lawson (UCB, U.K.) gave an inspiring talk on "Antibodies as Tools in NCE Discovery". Analysis of current FDA-approved antibody therapeutics clearly demonstrates the attraction that such drugs have in both medical treatment and market sales. Biologicals are forecast to exceed  $> 50\%$  of all drug sales, with Humira<sup>®</sup> predicted to generate USD 12.3 billion by 2018. However, the picture was very different in 1984, where no approved antibody therapeutics existed. As drugs, antibodies fulfill the critical remit of modulating PPIs, providing specificity and functional activity both in vitro and in vivo. However, due to their high molecular weight they must be delivered either by intravenous (i.v.) injection or subcutaneously (s.c.). In addition  $> 90\%$  of potential intracellular and CNS targets are not accessible to antibodies. Other potential challenges surround immunogenicity, and complex and costly manufacture, including analysis and supply chain logistics. From the antibody platform, added value applications have materialized, including targeted drug conjugates, target cross-linking and bispecifics. As an innovative strategy, UCB has embarked on developing a new generation of orally available drugs which incorporate the positive characteristics of antibodies. To this end, they are integrating antibody research with small-molecule NCE research, with the former providing tools to open up and provide enabling insight into new avenues of drug discovery exploration (5). In vivo profiling of affinity-matched murinized anti-mouse IL-6 research tools against the gp130 and gp80 axes clearly demonstrated the former as the preferred target for therapeutic intervention. Apart from aiding the development of the anti IL-6 MAb olokizumab, the information provided insight into the IL-6::gp80 dimer as a potential target to prosecute small-molecule fragment screening, as eloquently described from the lecture delivered by Dr. Rich Taylor from UCB. Antibodies also provide insight into intracellular processes as intrabodies and chaperone functions in structural studies. One exciting area resides in using antibodies to define specific conformations of proteins that could provide new and previously unexplored opportunities for intervention with small molecules. There is increasing literature, in particular

in the G protein-coupled receptor (GPCR) field, demonstrating the power of antibodies in stabilizing specific conformations, with the prospect of using powerful molecular dynamics simulation to reveal intermediate conformations (6-9). As a further example of UCB's technology and strategy, a small-molecule fragment screen against an antibody-stabilized target was described in which, using biacore technology, a number of hits have been identified. Interestingly, different stabilizing antibodies appeared to generate different hit collections. Several prioritized hits are currently undergoing Fab-enabled X-ray crystallography to define structural information.

#### CHALLENGES AND OPPORTUNITIES IN ALLOSTERIC ENZYME INHIBITION

Dr. Wolfgang Jahnke (Novartis Institutes for Biomedical Research, Basel, Switzerland) described three case studies of the challenges and opportunities in allosteric enzyme inhibition. In his first example—"allostery by design"—he described efforts to discover allosteric inhibitors of Abl kinase. Abl kinase is the target of imatinib and nilotinib, well-known treatments for chronic myeloid leukemia (CML). Both drugs are ATP-competitive inhibitors, and as such, slow development of resistance is sometimes encountered in the clinic due to mutation of Abl kinase in regions close to the ATP-binding site. An alternative "myristate" (Myr) binding site was identified by the Novartis Institute of Genomics Research (10). A subsequent fragment-based NMR screening campaign for Abl/Myr ligands, with the ATP site blocked by imatinib, resulted in 30 hits. Although the compounds lacked in vitro biochemical activity, structural work was highly successful, with nine of nine fragments delivering an Abl bound X-ray structure, showing binding to the Myr site. Analysis of this structural information enabled the team to hypothesize that fragments would gain functional activity via stabilization of the assembled Abl inactive conformation. Specifically, the fragment hits were structurally extended with the intention of driving the displacement of helix 1 towards the inactive conformation. At this point, the project was nearly terminated due to the lack of cellular activity following this approach; however, the team decided to develop a conformational assay for the displacement of helix 1. It was discovered that, in the presence of various Myr binding ligands, Val525 gave a very strong peak by HSQC NMR when helix 1 was unstructured and flexible, whereas when structurally well-ordered, only a small peak was observed. Intriguingly, compounds which produced the more ordered structure for helix 1 were also those that demonstrated functional activity, a prerequisite for project progression. This novel assay, followed by an optimization program, eventually resulted in the discovery of an allosteric BCR-ABL inhibitor currently in development at Novartis (<http://www.novartis.com/investors/event-calendar/recent.shtml>). In a preclinical blast crisis CML xenograft model, the combination of nilotinib and the allosteric inhibitor prevented the emergence of resistance, which was maintained for 50 days post-dosing.

In the second example, "allostery by selection", novel serine/threonine-protein kinase PAK 1 inhibitors were sought to provide cellular active compounds with improved kinase selectivity profiles. An analysis of known ATP-competitive inhibitors was performed, comparing their activity against non-phosphorylated versus phosphorylated PAK 1, which showed a clear differentiation between type I and type II kinase inhibition. In particular, type I inhibitors were less

potent against the unphosphorylated PAK 1 kinase domain, while type II inhibitors were more potent. A fragment-based screening NMR campaign was undertaken and efforts focused on validating hits which lacked the typical ATP hinge binding features. This was followed by substructure searching of the Novartis archive and analysis of the results of previous biochemical screening efforts. This led to the discovery of the dibenzodiazepine inhibitors, which were notable for their unusual biochemical profile. They were ATP-competitive and could be displaced by type I inhibitors, yet in the  $\pm$  phosphorylation assays they demonstrated typical behavior of type II inhibitors. Careful selection of appropriate molecules for crystallographic studies was then rewarded, with structural data revealing a novel binding mode (type III) for these molecules (Fig. 1).

The availability of this structural information enabled optimization of the dibenzodiazepines to give potent and selective PAK 1 inhibitors with greatly improved selectivity over previously available ATP-binding site inhibitors (Fig. 2).

Careful analysis of fragment screening hits and selection of analogues for crystallographic follow-up were needed to identify the first allosteric inhibitors of PAK 1 kinase. These inhibitors are potent, cell-permeable and selective for PAK 1, even over other PAK isoforms.

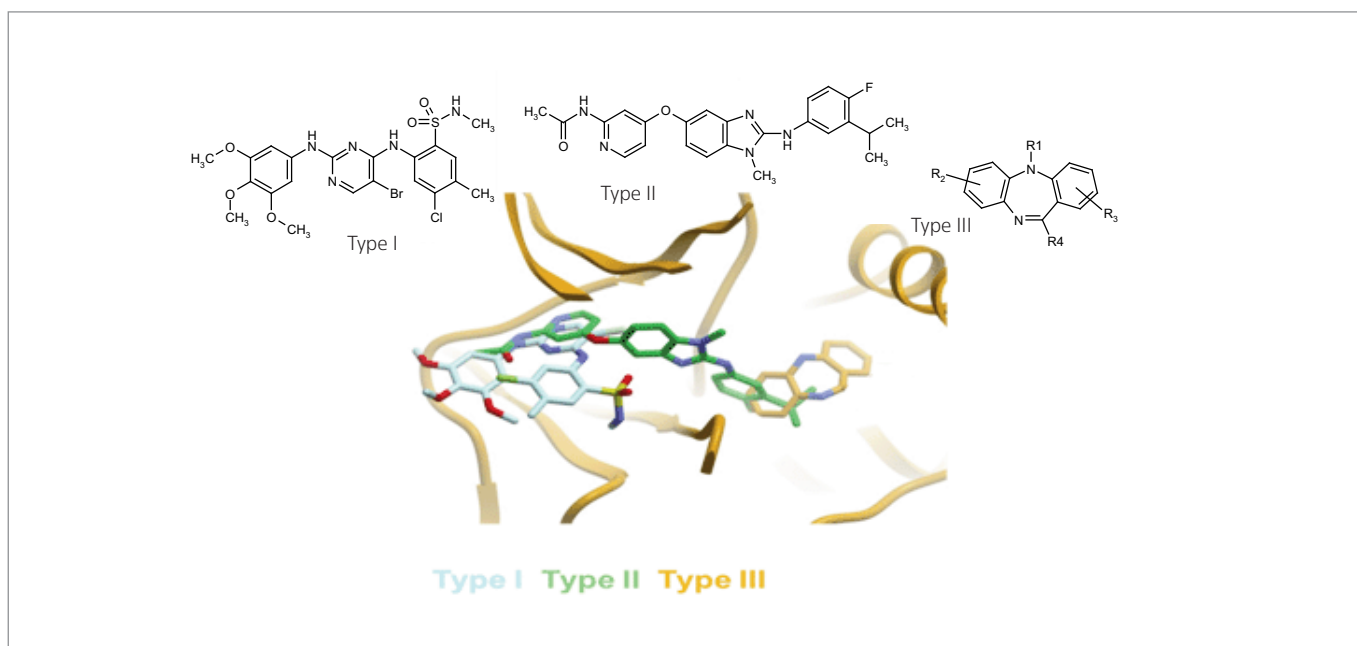
In the third example (allostery by serendipity), the discovery of allosteric inhibitors of farnesyl pyrophosphate synthase (FPP synthase) was described (11). Bisphosphonates are potent inhibitors of FPP synthase and are highly efficacious in the treatment of bone diseases such as osteoporosis. The potential for direct antitumor effects has been postulated and demonstrated clinically in early breast cancer patients treated with the potent bisphosphonate zoledronic acid (12). However, the high affinity of bisphosphonates for bone mineral

is suboptimal for the direct treatment of soft tissue tumors. Attempts to find alternative substrate-binding molecules lacking the problematic bisphosphonate structure have been unsuccessful.

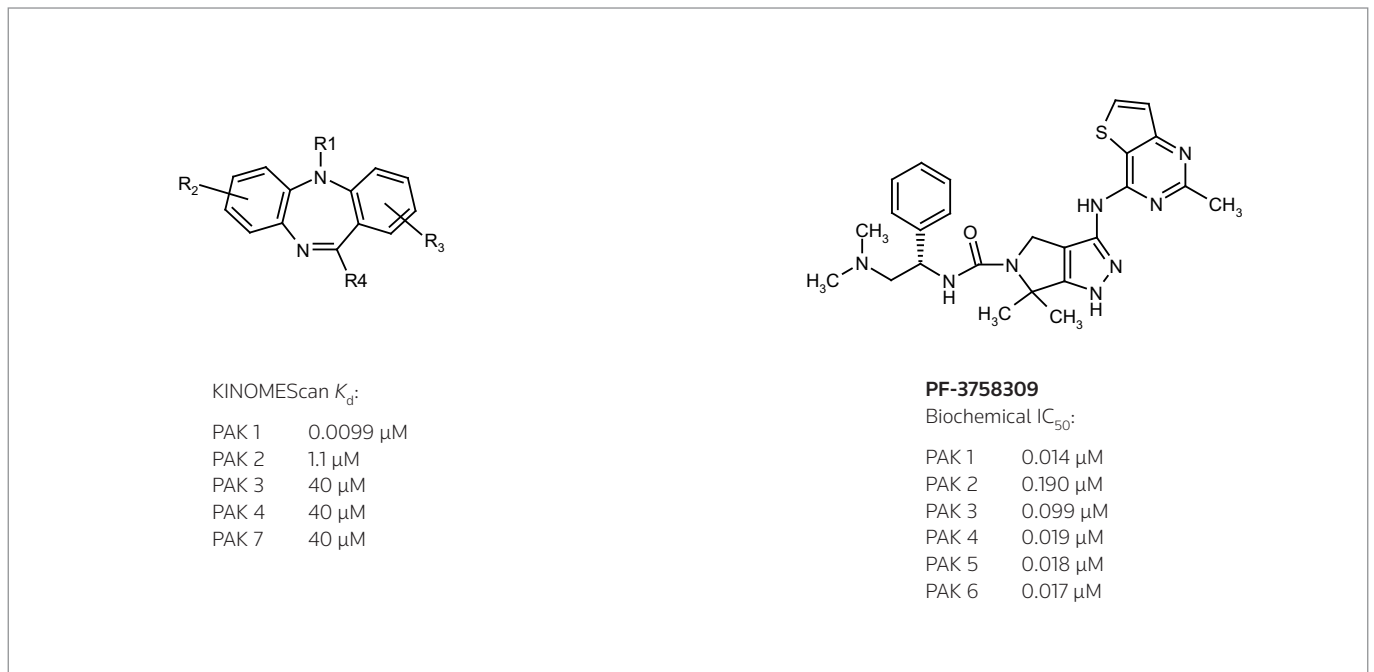
NMR fragment-based screening (using T1 $\rho$ /water LOGSY ligand observation experiments) for non-bisphosphonate FPP synthase inhibitors resulted in several hits, which were non-competitive or even synergistic with the bisphosphonate zoledronic acid. X-ray structural work on several of these hits bound to FPP synthase demonstrated that they bound to a novel allosteric pocket. Optimization of two series, including fragment merging, led to nM inhibitors of FPP synthase which completely lacked the bone binding behavior of bisphosphonates (Fig. 3).

### PHENOTYPIC SCREENING APPROACHES FOR THE DISCOVERY OF NOVEL ANTIMALARIALS

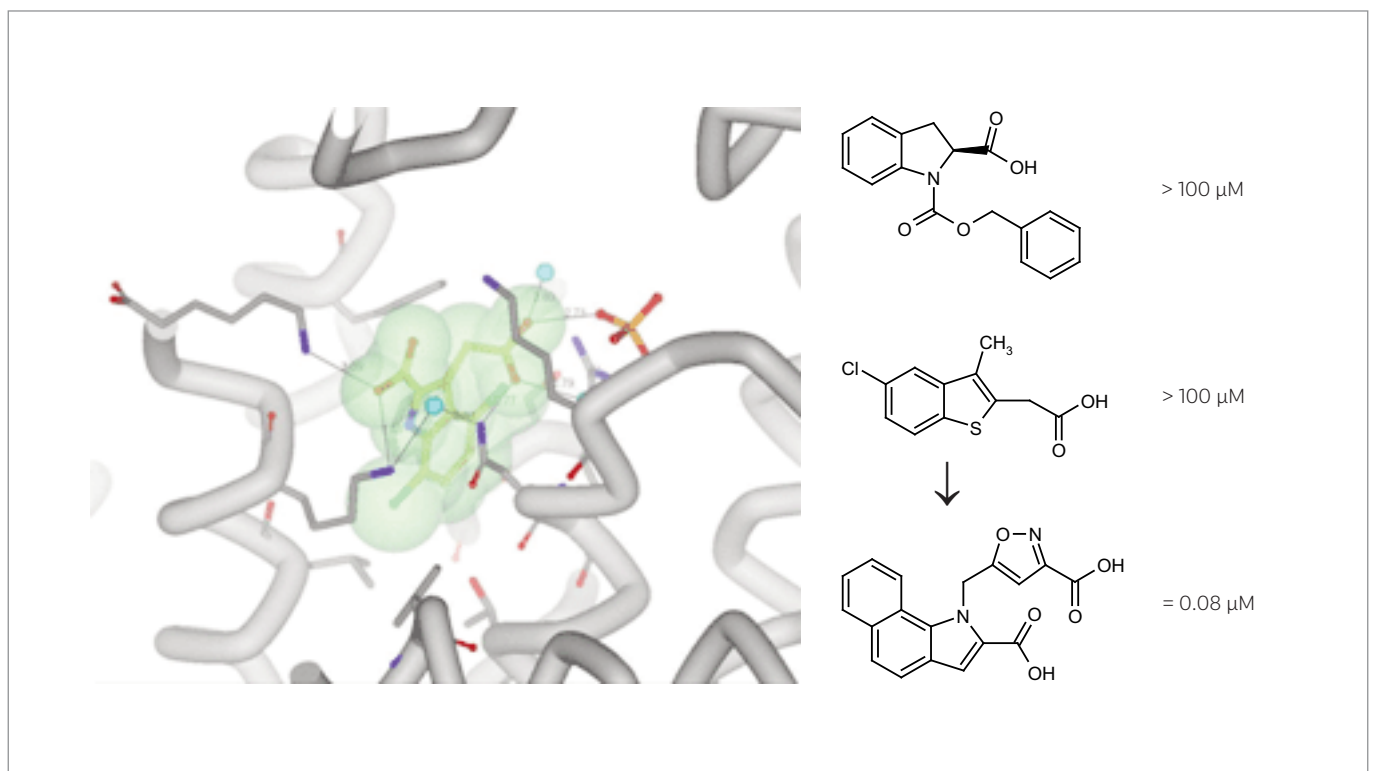
Dr. Javier Gamo-Benito (GlaxoSmithKline, Tres Cantos Medicines Development Campus, Diseases of the Developing World [DDW], Spain) discussed the challenges and opportunities of phenotypic screening for malaria. Malaria is a major global disease caused by parasites of the genus *Plasmodium*, with *Plasmodium falciparum* infection the single biggest cause of mortality. The recommended first-line treatments for *P. falciparum* malaria are artemisinin-based combination therapies (ACTs), although their effectiveness is now being compromised by resistance, with recent evidence indicating that slow-clearing *P. falciparum* infections have spread from Western Cambodia to the Thai-Burmese border. There is thus an urgent need for novel antimalarial drugs that can replace ACTs and provide future treatment options that have advantages over the current standards of care.



**Figure 1.** Inhibitor binding modes for the PAK 1 protein.



**Figure 2.** Differing kinase selectivity profile of PAK 1 inhibitors.



**Figure 3.** Binding mode and evolution of novel FPP synthase allosteric inhibitors.



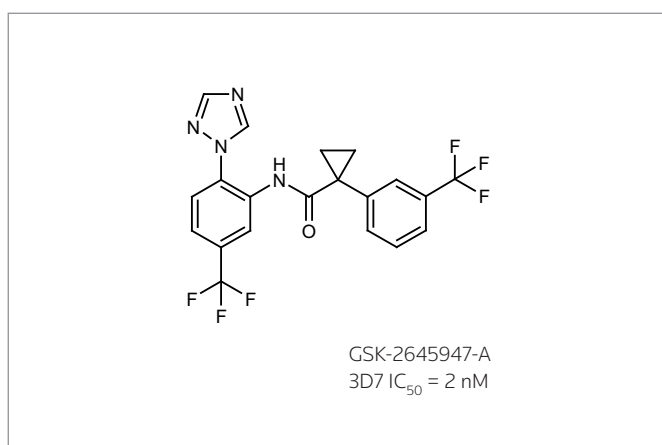
In order to discover new leads active in a whole-cell environment, an assay suitable for high-throughput screening was developed using *P. falciparum* intraerythrocytic stages of the parasite and *Plasmodium* lactate dehydrogenase (LDH) as a surrogate for parasite growth. After screening the GSK archive, hits were identified which showed > 80% parasite inhibition at 2  $\mu$ M. In 2010, GlaxoSmithKline (GSK) published the Tres Cantos antimalarial set (TCAMS) which comprises over 13,533 hit compounds derived from whole-cell screening of 2M compounds of the GSK corporate collection (<https://www.ebi.ac.uk/chemblntd>). This unique, freely available set offers an opportunity for the wider drug discovery community to enable the next wave of novel antimalarial agents.

Utilizing a series of functional assays and strains with resistance to known antimalarials, the Tres Cantos team filtered the antimalarial set to identify compounds with novel modes of action. These could be further prioritized based on parasite killing rates and gametocytocidal activity. One case study was described. A singleton hit from the TCAMS was selected and SAR studies allowed the identification of the cyclopropylcarboxamide **GSK-2645947-A** (Fig. 4). This compound had good oral bioavailability and outstanding oral efficacy; however, illustrating the challenges of malaria drug discovery, it showed a high propensity to select for resistance. Genomic experiments are ongoing to elucidate the molecular target for this series.

In conclusion, whole-cell screening has provided a wealth of new hits, shared widely with the antimalarial community, to support the discovery of novel antimalarials. Further profiling of TCAMS in liver stage and transmission blocking assays is anticipated to identify compounds with dual activity to enable the discovery of differentiated new antimalarials.

#### BICYCLES: HIGH-AFFINITY BICYCLIC PEPTIDES FROM PHAGE DISPLAY LIBRARIES

Dr. Christophe Bonny (Bicyclic Therapeutics, U.K.) opened his presentation outlining the objective of Bicyclic Therapeutics to identify bicyclic peptides with 1.5-2 kD molecular weight but with antibody-like properties. These peptides, stabilized as *tris*-thioethers, are



**Figure 4.** Representative member of the cyclopropylcarboxamide hit series.

highly constrained and cover a surface area of 400-1,000 Å. With the structural and physicochemical diversity that can be created with these bicyclic structures, they are ideally suited for targeting many PPIs that can be prioritized through bioinformatics analysis.

The approach is reliant on using phage display technology to build very large (up to 10<sup>12</sup> members) but rapidly screened libraries that include peptide diversity, variation in loop length and asymmetry, biased libraries with altered charge properties and structured libraries targeted at introducing defined structural motifs, particularly helical in nature. Following initial peptide synthesis, constraint is introduced using proprietary methodology to alkylate three obligate cysteines with 1,3,5-tris(bromomethyl)benzene or other thiophilic alkylating agents (13). To demonstrate the impact of the conformational constraint on affinity, a linear peptide was shown to be 100-fold less potent than a monocycle of the same sequence, while the corresponding bicycle was at least 10-fold more potent than the monocycle.

So far this approach has been successfully applied to more than 50 targets, with ligands routinely demonstrating subnanomolar affinities following optimization through new cycles of library generation and single mutations of high-affinity sequences to understand SAR. Furthermore, excellent selectivity can be achieved, for example, human plasma kallikrein compounds with a K<sub>i</sub> of < 1 nM were identified, which retained low nM rat kallikrein affinity but > 10  $\mu$ M affinity against human thrombin, coagulation factor XI or coagulation factor XII.

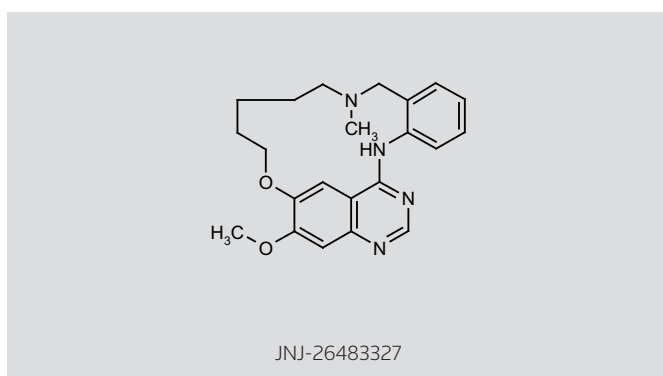
While bicyclics generated with natural L-amino acids do show proteolytic instability, plasma stability can be readily introduced by the use of non-natural amino acids. Furthermore, this same strategy introduces in vivo metabolic stability, as with an example of an all L configuration bicycle showing a clearance of 156 mL/min/kg in mice, whereas the optimized ligand had a clearance of 5.9 mL/min/kg and a t<sub>1/2</sub> > 3 hours. Conjugating to fatty acids or to the albumin binding peptide SA21 can further extend the half-life, while retaining target activity. Furthermore, 50% systemic exposure was reported for an example following intratracheal dosing, demonstrating high tissue penetration. While antibodies can access the vascular space, they tend not to be able to access the extravascular space; this space is seen as the natural home for bicyclic peptides with molecular weights < 5 kD (14).

The potential of bicyclic peptides in oncology exploiting the prospect of good tumor penetration, high affinity for the target and fast renal clearance to give high tumor/blood ratios was discussed. Furthermore, a cytotoxic agent, a radionuclide payload or a second bicyclic peptide (for targeting tissues or synergistic or complementary target pathways) can be conjugated to the bicyclic peptide. Dr. Bonny highlighted one oncology target, which is rapidly internalized, as an example where a highly selective subnanomolar peptide bicyclic inhibitor was detected within minutes within the lysosomes of a cell-based assay. Furthermore, in a mouse fibrosarcoma HT-1080 model the compound was shown to be rapidly taken up in tumors or excreted via renal clearance, in marked contrast to an antibody showing little distribution from the liver to the tumor. Further opportunities discussed for this technology included different routes of administration by optimizing physicochemical properties and by appropriate formulations for depot dosing.

## LEADING INTO NEW SPACES – STRATEGIES FOR LEAD GENERATION IN NEUROSCIENCE MEDICINAL CHEMISTRY

In discussing the work of the Janssen Neuroscience hit generation team, Dr. Rombouts (Janssen, Belgium) focused on macrocycles, fragment-based lead generation, scaffold hopping and the importance of high-quality synthetic chemistry.

Interest in macrocycles (15, 16) centered on locking the bioactive conformation of molecules, to modulate activity, selectivity and physicochemical properties, particularly related to CNS disposition, often a challenge for kinase inhibitors, and to generate new intellectual property space. Analysis of the epidermal growth factor receptor (EGFR) kinase crystal structure of gefitinib suggested an approach to macrocyclization, which resulted in the identification of **JNJ-26483327**, with excellent rat brain exposure (AUC = 2,347 ng.h/g) and a brain:plasma ratio of 1.6-2.0 at 10 mg/kg compared to gefitinib (brain:plasma ratio = 0.35).

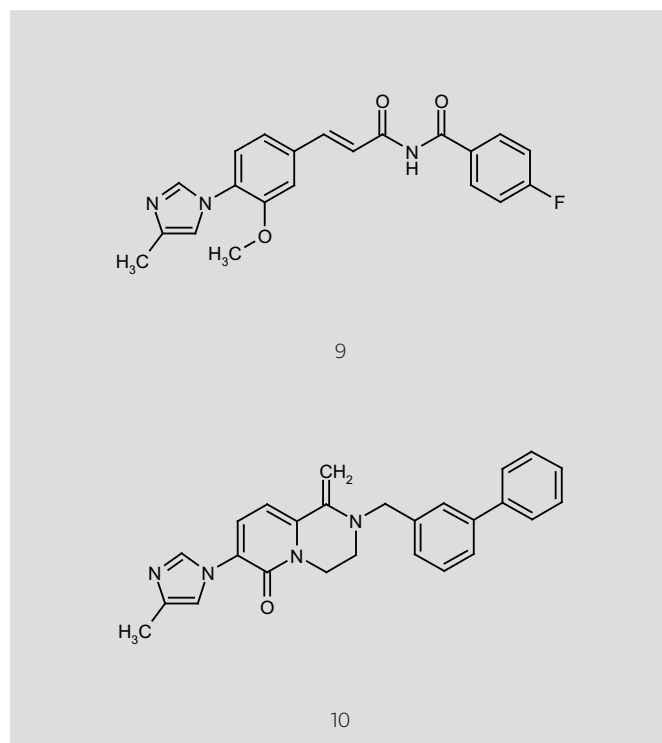


However, macrocyclization is not a universal panacea, as demonstrated with a series of macrocyclic  $\alpha 7$  positive allosteric modulators. While good target activity and tissue exposure could be achieved, increases in lipophilicity and rigidity tended to compromise solubility, with the macrocyclic linker a challenge to optimize. Overall, it was concluded that a good template profile is required to justify a macrocyclization strategy, starting with simple linkers —macrocyclization itself is unlikely to address problems inherent in the template.

The Janssen fragment-based screening library of 2,000 compounds was assembled from the internal collection containing between 8 and 21 heavy atoms, and with full QC and kinetic solubility > 200  $\mu\text{M}$ . This library was relatively more complex than commercial libraries, as determined by numbers of  $\text{sp}^3$  atoms and chiral centers. Screening of this library against phosphodiesterase PDE2 using biacore technology gave a hit fragment ( $\text{pIC}_{50} = 5.3$ ; LE = 0.35) that was confirmed, with a high-concentration surface plasmon resonance assay and ligand observed NMR/displacement studies. Optimization increased affinity 600-fold, although selectivity over PDE10 was only 4-fold. Exploiting a pocket identified in PDE2, but absent in PDE10, rapidly gave a compound with 65-fold improved selectivity that also

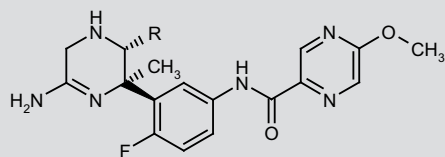
showed enhanced metabolic stability and was progressed into lead optimization studies.

Highlighting the role of excellence in synthesis for drug invention, examples of scaffold hopping and manipulation of  $\text{pK}_a$  were outlined, which both required significant synthetic chemistry input. Scaffold hopping on the cinnamide  $\gamma$ -secretase inhibitor **9** screening hit focused on removing the cinnamic acid and led to the potent pyrido[1,2-*a*]pyrazine **10**, in addition to opening up chemistry that allowed rapid synthesis of analogues.



A strongly basic amidine is key for an interaction with the aspartyl protease BACE, but also introduces major P glycoprotein efflux liability. A strategy was therefore devised to reduce the  $\text{pK}_a$  by de novo design of analogues, targeting compounds with a calculated  $\text{pK}_a$  range of 6.5-8. From this, a set of chiral 2-substituted-3,3-disubstituted-1,4-oxazines were targeted, of which the fluoro analogue **11** ( $\text{pK}_a = 7.8$ ) proved to have excellent BACE inhibitory potency, activity against  $\beta$ -amyloid ( $\text{A}\beta$ ) in cells, and unlike **12** ( $\text{pK}_a = 9.6$ ), it was not a P glycoprotein substrate. Further optimization of the amide gave compounds with excellent in vivo efficacy in lowering  $\text{A}\beta 40$  and  $\text{A}\beta 42$ . A key element of progress in this work was the academic collaboration that enabled the development of synthetic routes to key chiral precursors of the substituted oxazines (17). The benefits emphasized from these academic synthetic chemistry collaborations were the development of complementary expertise in, for example,





11 R = F

12 R = H

chiral induction and fluorine chemistry, enriching the Janssen compound collection with novel scaffolds and with post-docs spending part of their time in Janssen's labs introducing new expertise and ideas to the organization.

## DISCLOSURES

R. Porter is the owner of Rod Porter Consultancy. A.J. Ratcliffe and S. Collingwood are employees of Redx Anti-infectives and Novartis, respectively.

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