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MEETING REPORT

TRENDS IN MEDICINAL CHEMISTRY

HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM HELD ON DECEMBER 9, 2010 AT NHLI, LONDON, UK

P. Cowley[1], N. Hales[2] and S. Ward[3]

[1]TPP Global Development, Edinburgh, UK; [2]Allodigm, Macclesfield, Cheshire, UK; [3]University of Sussex, Brighton, UK

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SUMMARY

On December 9, 2010, the Society for Medicines Research held a one-day meeting entitled "Trends in Medicinal Chemistry". This symposium, organized by Phillip Cowley, Neil Hales and Simon Ward, brought together a panel of international speakers to present and discuss the advances that are currently being made. In recent years, there have been significant changes to the landscape of the pharmaceutical industry. The aim of this meeting was to allow scientists within drug discovery programs in biotech, pharma and academia to be updated on new methodologies and screening approaches, as well as capture best practices from key case study presentations.

Correspondence: Society for Medicines Research – Secretariat, 840 Melton Rd., Thurmaston, Leicester LE4 8BN, UK. E-mail: secretariat@smr.org.uk.

CALORIMETRY AS A TOOL FOR UNDERSTANDING BIOMOLECULAR INTERACTIONS AND AN AID TO DRUG DESIGN

Professor John Ladbury (Department of Biochemistry & Molecular Biology, MD Anderson Cancer Center, Houston, TX, USA) described the complexity of the binding of two biomolecules from the atomic level. This process involves the formation, or removal, of many individual noncovalent bonds between both the interacting molecules and solvent. Professor Ladbury stated that our current understanding of the quantification of this process on the thermodynamic level is somewhat naive.

Isothermal calorimetry (ITC) provides a rapid route to full thermodynamic characterization of a biomolecular interaction. Direct determination of the change in enthalpy (ΔH) for complex formation and the use of this to probe the extent of an interaction through a titration enables the calculation of the equilibrium binding constant (K_B). With these terms, a full thermodynamic characterization of the interaction can be determined using Equation 1.

$$\text{Equation 1: } -RT \ln K_B = \Delta G = \Delta H - T\Delta S$$

where R is the gas constant, T is the absolute temperature, ΔG is the change in free energy ongoing from free (unbound) to associated (bound) states and ΔS is the change in entropy.

Professor Ladbury then explored the use of these data to improve our understanding of complex formation and provide tools for drug discovery. The SCORPIO (Structure-Calorimetry of Reported Protein Interactions Online database) provides a useful set of data derived solely from ITC experiments, published in peer-reviewed journals and subsequently checked (1, 2). Upon analysis of the full dataset, the change in free energy for all interactions showed a moderate correlation with total buried surface area (r^2 = 0.62) and apolar buried surface area (r^2 = 0.65). Complexes burying more apolar surface area bound with higher affinity. However, the relationship between ΔG and buried apolar surface, contrary to common belief, could not

be simply explained by an underlying correlation with TΔS. The situation is clearly more complex.

Thermodynamic data for binding of a series of ligands to the Src homology 2 (SH2) domain were used to illustrate the utility of determining ΔH during optimization. Variation of an amide substituent provided a series of ligands with similar values for ΔG, but broad variation in the ΔH and TΔS components that made up the overall change in free energy. The speaker then introduced the concept of enthalpic efficiency as a parameter for compound selection (Equation 2).

Equation 2: Enthalpic efficiency (EE) = ΔH / N

where N is the number of non-hydrogen atoms or molecular weight.

The speaker then highlighted data from a recent publication, on which he was a coauthor, suggesting that for both statins and HIV protease inhibitors the "best-in-class" compounds were those that showed the largest ΔH contribution to the free energy of binding (3). This supported the proposition that measurement of ΔH and calculation of enthalpic efficiency could enable decision making in drug discovery programs, leading the medicinal chemist towards higher-quality drug candidates.

BIOPHYSICAL TECHNIQUES IN DRUG DESIGN – A MEDICINAL CHEMISTRY PERSPECTIVE

Dr. David Millan (Pfizer, Sandwich, UK) described recent progress at Pfizer in the use of biophysical techniques in drug discovery. He advocated looking "beyond the IC_{50}" using kinetics, thermodynamics and analysis of binding modality. The key biophysical techniques employed are X-ray, nuclear magnetic resonance (NMR), surface-plasmon resonance (SPR) and ITC.

The speaker highlighted the importance of kinetics in binding events, where $K_D = k_{off}/k_{on}$. The dissociation rate constant (k_{off}) relates purely to the lifetime of the bound state, and decreasing k_{off} increases the half-life of this state. As the K_D relates only to the ratio of the association and dissociation rates and not the absolute values, it follows that compounds with the same affinity can possess very different kinetics. These parameters are measured using SPR.

The use of binding kinetics in drug design was illustrated using the discovery of inhaled mitogen-activated protein kinase p38 inhibitors for the treatment of chronic obstructive pulmonary disease (COPD), which were intended to have slow offset kinetics to enable once-daily dosing. Previously described p38 inhibitors include VX-745 and BIRB-796 (doramapimod) (Fig. 1). While both of these compounds show a K_D of 1 nM, the kinetics are very different, leading to $t_{1/2}$ values of 30 seconds and 23 hours, respectively. VX-745 binds at the ATP site in a DFG-IN mode, leading to fast association and dissociation. BIRB-796 binds to the pocket created by DFG-OUT loop movement, and then enters the ATP site, giving slow association and dissociation. The Pfizer project hybridized CE-159167 (known to have fast on/off kinetics) and BIRB-796 using crystal structure overlays. This led to the discovery of PF-994888, with a K_D of 0.1 nM and slow offset kinetics, subsequently confirmed to be a result of taking up the DFG-OUT binding mode.

The use of thermodynamics in drug design was illustrated using a recently published example, based around the discovery of carbonic anhydrase inhibitors (4). Thermodynamic screening of a set of benzenesulfonamides (BSAs) provided compounds with a range of thermodynamic profiles. As the substitution pattern on the BSA varied, significant differences were noted in the ΔH component of binding energy. In particular, the 2-F BSA was noted to have enthalpically

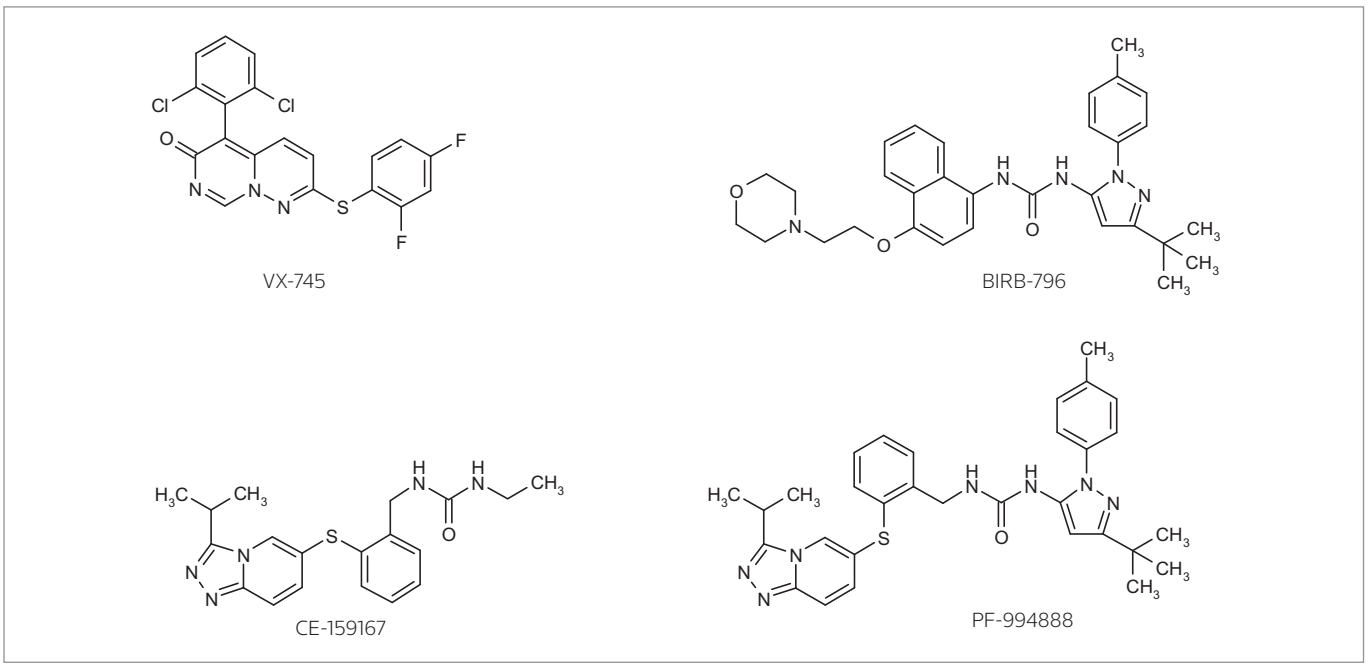


Figure 1. Structures of VX-745, BIRB-796, CE-159167 and PF-994888.

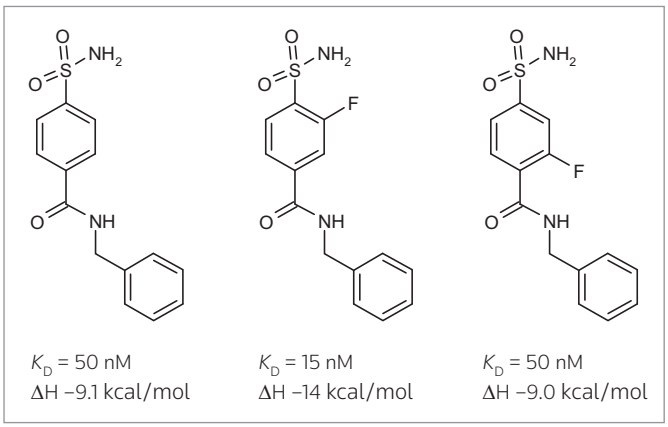


Figure 2. Structures and thermodynamic signatures of benzenesulfonamides.

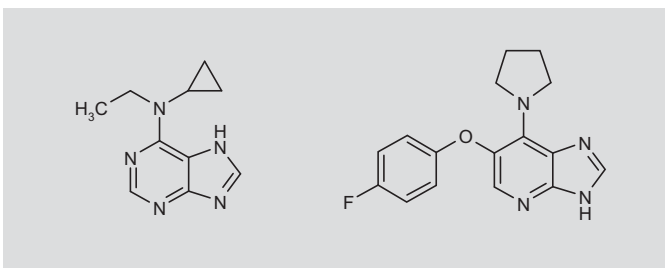
driven binding brought about by an interaction between the fluorine atom and the protein backbone. Interestingly, the relative thermodynamic signatures were retained when the fragments were grown into the compounds exemplified in Figure 2 by addition of a benzyl amide, with the 2-F BSA exhibiting a ΔH component of –14 kcal/mol.

THE STRUCTURE-BASED OPTIMIZATION OF ORALLY ACTIVE METAP 2 INHIBITORS FOR THE TREATMENT OF INFLAMMATION

Dr. Nigel Parr (GSK, Stevenage, UK) described a successful structure-based optimization of HTS hits to achieve potent methionine aminopeptidase 2 (MetAP 2) inhibitors with use in the inhibition of antibody secretion by B-cell-derived plasma cells.

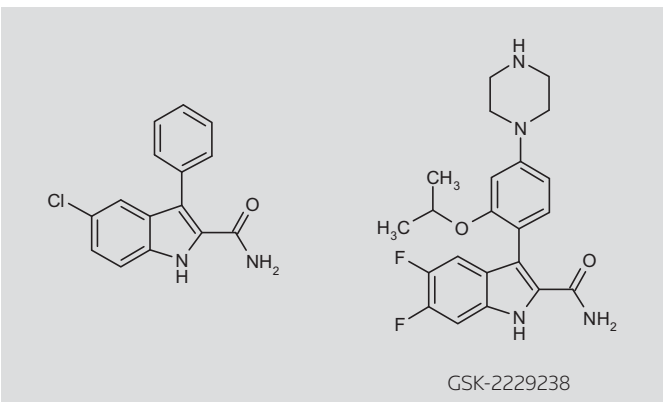
MetAP 2 cleaves protein *N*-terminal methionine to leave an *N*-terminal glycine, which can then be, e.g., myristoylated. Dr. Parr highlighted the importance of MetAP 2 in the maturation of cells of the B-cell lineage. Because such cells are involved in the secretion of antibodies during inflammatory processes, MetAP 2 inhibitors should act as antiinflammatory agents. This hypothesis is supported by results with the clinical antiangiogenic agents fumagillin and ovacilin, which showed in vivo efficacy in a range of animal models of inflammation, but unfortunately, their use is contraindicated due to toxicity.

An HTS identified four series of MetAP 2 inhibitors and Dr. Parr described the optimization of the two series, which showed specificity versus MetAP 1, Lipinski-compliant molecular properties, an established protein–ligand crystal structure and promising pharmacokinetics.



The first series, based on 6-(*N*-ethyl-*N*-cyclopropylamino)adenine (MetAP 2 pIC_{50} = 7.0), was elaborated with the guidance of structural information on the 5-(4-fluorophenoxy)-6-pyrrolidinoimidazolo[4,5-*b*]pyridine (MetAP 2 pIC_{50} = 8.5) and its analogues. The potency of the compounds was now approaching the tight-binding limit, so that ranking was difficult and work on *Escherichia coli* MetAP (Qi-Zuhang Ye) showed that the enzyme needed only one Mn(II) metal ion rather than the two previously thought. This meant that the chosen in vitro assay system needed to be rethought. Despite potency against the enzyme, compounds of the imidazolopyridine series had poor activity in the cell-based assay. The problem was not due to permeability, solubility or stability, but to compromised in vitro data and lack of cellular activity, and work turned to the second series.

The second series, based on 5-chloro-3-phenylindole-2-carboxamide (MetAP 2 pIC_{50} = 6.5), was elaborated with the guidance of structural information and a knowledge of what had worked previously. Introduction of an additional 6-halogen to improve shape complementarity, introduction of an *O*-alkoxy group on the phenyl ring to extend binding into a nearby "shelf-region", and the introduction of a piperazine ring on the *para*-position of the phenyl ring to improve solvent exposure and solubility all improved potency. The combined effect of all of these changes was seen in the difluoro-isopropoxy-piperazine analogue GSK-2229238 (MetAP 2 pIC_{50} = 8.7; MetAP 1 pIC_{50} < 4.6; human tonsillar B-cell assay pIC_{50} = 7.1). These compounds clearly showed that no useful cellular activity (human tonsillar B-cell assay pIC_{50} > 5.3) was likely to be seen if the MetAP 2 pIC_{50} was < 8.0.



At 30 mg/kg b.i.d. GSK-2229238 produced trough blood levels near the B-cell assay IC_{50} (adjusted for fraction unbound; HSA binding 96%), and at this dose b.i.d. in vivo, TNP-specific IgG was reduced 89%. There was a corresponding reduction in CD23 expression in treated animals and ex vivo splenocyte proliferation from treated animals was also inhibited, consistent with an impact on the B-cell population.

Overall, Dr. Parr demonstrated successful structure-based optimization of HTS hits to achieve highly potent MetAP 2 inhibitors that

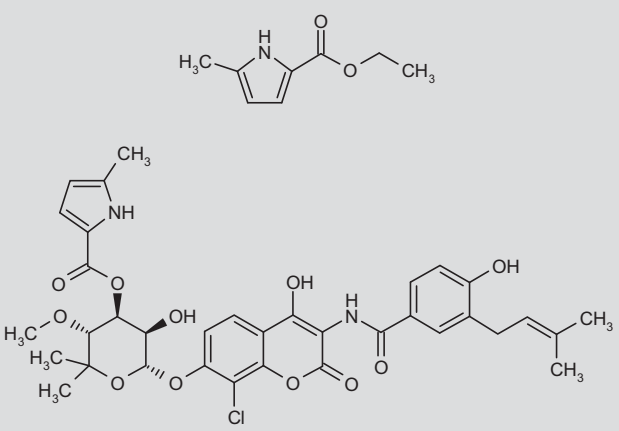
exerted intracellular target inhibition. A representative compound was orally active in vivo and pharmokinetic data indicated that maintaining > 50% target inhibition drives pharmacodynamics such that reversible inhibitors of MetAP 2 inhibit antigen-specific antibody production in a mouse immunization model, with > 80% inhibition of the T-cell-dependent antibody response. Reversible MetAP 2 inhibitors therefore represent an exciting class of therapeutics for the treatment of immune inflammatory conditions.

NOVEL DNA GYRASE INHIBITORS: FRAGMENT-BASED NMR SCREENING TO ANTIBACTERIAL AGENTS

Dr. Ann Eakin (AstraZeneca, Boston, MA, USA) described the application of topical biophysical techniques used in the discovery and development of a novel series of antibacterial agents.

Dr. Eakin highlighted the increased prevalence of resistant bacteria to essentially all of the marketed antibacterial agents, whatever their mechanistic class. Bacterial DNA replication is significantly different from its mammalian counterpart, with the quinolone antibacterials targeting DNA gyrase and topoisomerase IV, the only topoisomerase validated by current drugs in the clinic. The aim of the work described was to discover novel agents that target DNA gyrase with a distinct mechanism of action, to avoid cross-resistance with existing drugs. The target chosen was the ATP binding site of the DNA gyrase subunit B (gyrB) protein.

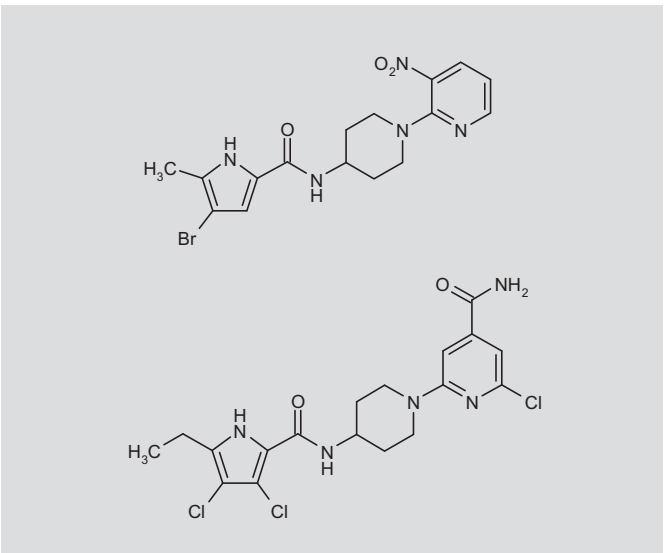
Useful low-molecular-weight "fragments" with binding affinity for gyrB were identified as starting points by NMR screening, which highlighted ethyl 5-methylpyrrole-2-carboxylate (K_d = 1,070 μM), a fragment of the potent natural product gyrase inhibitor clorobiocin.



Clorobiocin

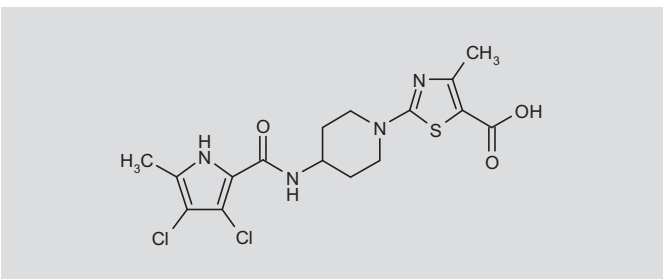
A second round of screening identified 5-nitro-8-hydroxyquinoline-7-carboxylic acid (K_d = 5,000 μM) as a ligand for an adjacent site. The chemical approach taken was to elaborate the pyrrole carboxylate ligand in the direction of the adjacent quinoline site using a structure-based design.

Medicinal chemistry established that pyrrole amides could replace the pyrrole esters, and then a directed library of 100 compounds was synthesized, with emphasis on simple chemistry, structural diversity and desirable physicochemical properties. The combination of a bromopyrrole with a nitropyridine gave a useful enzyme inhibitor (*E. coli* gyrase ATPase IC_{50} = 0.61 μM; K_i = 0.28 μM), but the compound lacked antibacterial activity and had a high microsomal clearance. The binding mode was shown by crystallography to be as expected.



Further elaboration of pyrrole and pyridine moieties gave the ethylpyrrole derivative shown, which was not only a more potent inhibitor of *E. coli* gyrase ATPase (IC_{50} = 0.01 μM) and *S. aureus* gyrase ATPase (IC_{50} = 0.03 μM), but also showed antibacterial activity against the clinically important pathogens *Streptococcus pneumoniae* (MIC = 1 μg/mL) and *Staphylococcus aureus* (MIC = 4 μg/mL), with satisfactory human microsomal clearance (< 14 μL/min/mg).

Further variation to improve physicochemical properties gave compounds with desirable plasma levels following oral doses to mice that provided suitable exposure to support efficacy studies. A thiazole 4-carboxylic acid analogue in the pyrrole amide series reduced colony-forming units of *S. pneumoniae* in mouse lungs by > 4 log compared to vehicle-treated control at 320 mg/kg/day b.i.d. or q.i.d.



Dr. Eakin showed that pyrrole amide analogues block DNA synthesis through inhibition of DNA gyrase in cells. These compounds are bactericidal, with low frequencies of resistance (< 1 x 10^{-8} across bacterial species), and also maintain potency against drug-resistant bacterial strains (Table I).

Overall, Dr. Eakin demonstrated that the pyrrole amides are potent inhibitors of the ATPase and supercoiling activities of DNA gyrase. The series demonstrates promising antibacterial activity across a broad spectrum of pathogens, and the compounds are bactericidal and demonstrate a cellular mode of action of DNA synthesis inhibition. The series contains compounds that combine pharmacokinetic and physical properties suitable for oral dosing, and members of the series showed efficacy in an *S. pneumoniae* lung infection model in mice. Consequently, the pyrrole amide series is a novel class of antibacterials with potential to deliver drugs that target multiple clinical indications.

MINIATURIZED PROTEIN THERMAL STABILITY MEASUREMENTS: FROM HTS TO LEAD OPTIMIZATION

Dr. Matthew Todd (Johnson & Johnson, Spring House, PA, USA) described the utilization of ThermoFluor® within his organization. This technology is a miniaturized protein thermal stability measurement used for the discovery of a compound's ability to bind to a therapeutic target protein. Small, predictable changes in protein melting temperature in the presence of equilibrium binding ligands allow an accurate assessment of compound affinity. The technology utilizes a fluorescence readout, caused by interaction of the protein with a dye upon reaching the melting temperature. Protein stability is increased by the binding of ligands. ThermoFluor® provides a direct, biophysical binding assay with broad applicability to enzymes and receptors, although membrane proteins present unique challenges. Assay development is rapid and the technology has been shown to correlate well with secondary assays. Within Johnson & Johnson, ThermoFluor® is used to generate protein stability profiles (providing data on protein preparation, crystallography and formulation), to carry out HTS and for hit profiling.

As an HTS technology, in combination with automation, ThermoFluor® can carry out over 50,000 melt experiments per day. A screening campaign against lipid hydrolase was described in which ThermoFluor® provided the initial binding assay; screening positives were then followed up in a fluorescence-based assay of enzyme activity using a nonphysiological substrate.

ThermoFluor® can then be used for hit profiling, initially including calculation of binding constants. Competition studies can be used to discriminate binding at allosteric sites from active sites. Off-target analysis can also define compound mechanism of action, as some active compounds may act nonspecifically as protein denaturants. A triage of "bad compounds", which perturb the assay results through dye binding or protein destabilization, was also highlighted using a screening campaign against tyrosine-protein phosphatase 1B (PTP-1B) to illustrate the points.

Table I. *Bactericidal activity of pyrrole amide analogues.*

Bacteria	Number of strains	MIC value	MIC (μg/mL)
Staphylococcus aureus	29	MIC_{90}	2
MRSA	14	MIC range	1-2
Enterococcus spp.	30	MIC_{90}	1
VRE	9	MIC range	0.5-1

MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*.

SELECTIVE GLYT1 INHIBITORS: DISCOVERY OF RG-1678, A PROMISING NOVEL MEDICINE TO TREAT SCHIZOPHRENIA

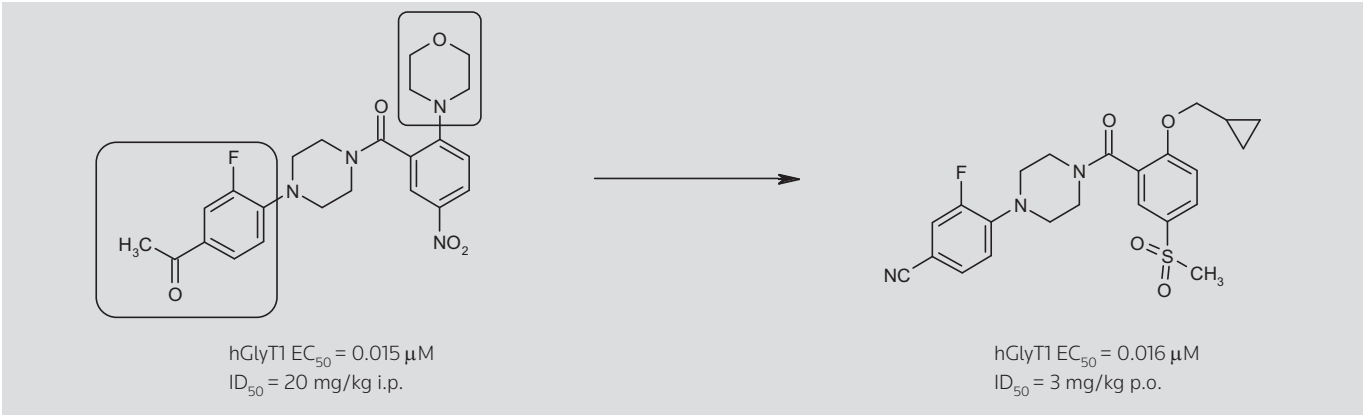
Emmanuel Pinard (F. Hoffmann-La Roche AG, Basel, CH) described the discovery program that led to RG-1678. Dr. Pinard highlighted the background of the disease, currently controlled by dopamine receptor D_2 antagonists, such as olanzapine. While able to manage the positive disease symptoms, drugs like olanzapine are less effective against the negative symptoms and cognitive deficits. Therefore, the need to explore alternative approaches, such as improving glutamatergic neurotransmission, is necessary. The sodium- and chloride-dependent glycine transporter 1 (GlyT1) transporter maintains glycine at subsaturating levels at the glycine binding site, and, as glycine is an obligatory co-agonist for the NMDA channel, elevation of glycine levels through GlyT1 inhibition could potentially address the NMDA receptor hypofunction thought to underlie the schizophrenic disease state.

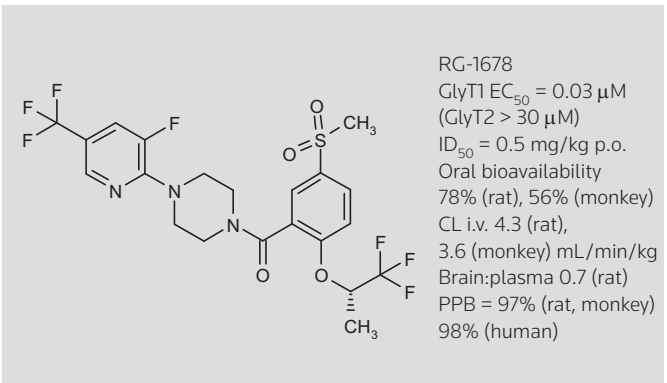
Research efforts at Roche were initially unsuccessful across multiple chemical series; however, a second HTS identified the starting point that ultimately delivered the candidate. This hit was a potent GlyT1 inhibitor, but required optimization, in particular to improve metabolic stability and remove the nitro group. Initial efforts reported in 2008 identified the sulfone as the best replacement, which improved permeability, solubility, metabolic stability and GlyT2 selectivity. Further exploration of the morpholine and acetophenone groups uncovered a wide diversity of tolerated functionality. Alkoxy groups, such as those shown on the next page, were superior to amino, aromatic and alkyl groups for overall solubility, metabolic stability and oral activity. To maximize the throughput of in vivo characterization, a pharmacodynamic model was developed in mice for reversal of L-687414 (NMDA glycine-site antagonist)-induced hyperlocomotion. This model could screen up to 20 compounds/week and correlated well with the increases in glycine level seen on microdialysis.

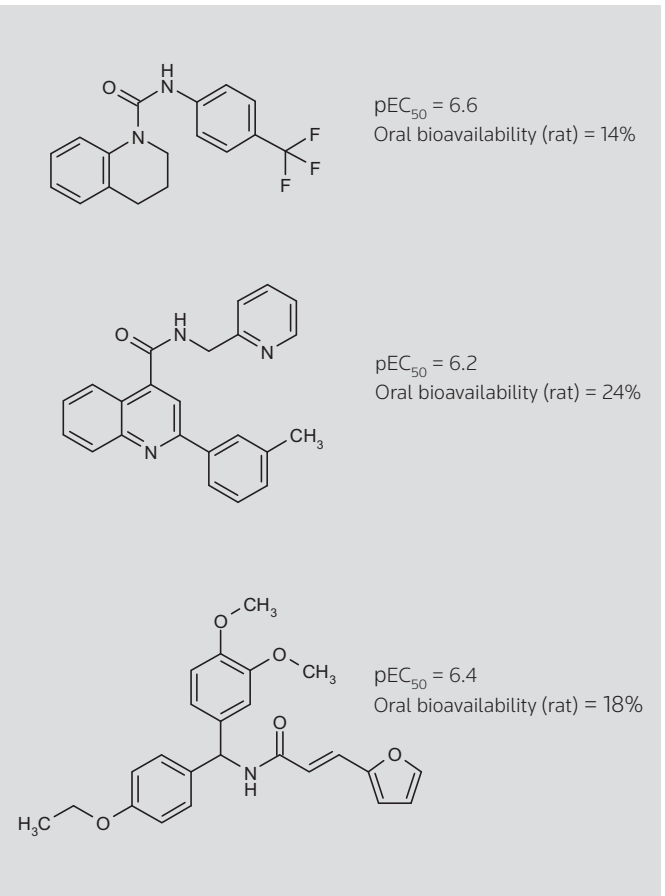
Further optimization was needed to improve CNS penetration and reduce hERG channel inhibition. This was accomplished by modification of the alkoxy substituent to include the CF_3 group (*S*-enantiomer most potent). Further modification of the cyanophenyl to pyridyl group led to further improvement in hERG, and retaining the fluoro group on the pyridine helped maintain CNS penetration, leading to candidate RG-1678. This molecule was profiled in an initial phase II trial in 320 patients (10, 30 and 60 mg once daily for 8 weeks), showing that the compound improved both the negative symptoms and the personal and social functioning of patients with schizophrenia, reaching statistical significance on primary and secondary endpoints.

NOVEL ALLOSTERIC MODULATORS OF THE NICOTINIC ACETYLCHOLINE α7 RECEPTOR

Dr. Veronique Birault (GlaxoSmithKline) described their program to identify positive allosteric modulators of the nicotinic acetylcholine







α7 receptor, thought to be implicated in the pathophysiology of both schizophrenia and Alzheimer's disease. Allosteric modulators were targeted to minimize the risk of tolerance and preserve the spatial and temporal pattern of receptor potentiation. An HTS delivered four hit series (three shown in the righthand column) and one singleton, and Dr. Birault described computational maps of CNS and oral drug space which were used to ensure that the hit series and subsequent analogues were of high quality.

The first series was optimized to improve pharmacokinetics and avoid the genotoxic anilinic fragment; however, solubility was poor. This work ultimately delivered a program from an undisclosed chemical series that moved into full lead optimization and subsequently delivered a clinical development candidate that was effective in behavioral models (novel object recognition, auditory gating), with an acceptable safety profile in rats and dogs.

DISCLOSURES

Dr. Phillip Cowley is Head of Chemistry at TPP Global Development, Edinburgh, U.K. Dr. Neil Hales is Medicinal Chemistry Consultant at Allodigm and a research fellow in the Department of Pharmacy at the University of Manchester. Professor Simon Ward is Director of the Translational Drug Discovery Group and Professor of Medicinal Chemistry at the University of Sussex, Brighton, U.K. The SMR would like to thank Aptuit, Optibrium, Ltd. and Activate Scientific for sponsorship of the meeting. The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year. These one-day conferences are multidisciplinary in nature and focus on various aspects of medicines research. Details of forthcoming meetings can be found at http://www.smr.org.uk or by e-mail to secretariat@smr.org.uk.

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