Molecules that matter: case studies in medchem

In a two-part review article, Corinne Kay of Med-Simple summarises research described in Scientific Update's and Select Conferences' jointly organised MedChem Europe conference held during this year's BioFine event in Berlin, Germany in April.

The MedChem Europe Conference jointly organised by Scientific Update and Select Conferences and held as part of avakado's BioFine event from April 13-14, 2005 at Messe Berlin, Germany attracted a high-quality audience from around the world. The following summarises the ground-breaking research presented at the conference.

Research into NNRTI drugs

The first presentation at MedChem Europe was given by Dr Bart DeCorte of Johnson & Johnson Pharmaceutical R&D. This consisted of a comprehensive overview of the HIV virus replication cycle and an historical account of the discovery of Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI), which started at Johnson & Johnson in the 1980s.

The research was heralded by the early discovery of TIBOs following a directed screening campaign. TIBOs were the first NNRTIs active against wt HIV-1 virus. Two additional, promising classes of NNRTIs were then identified: a-APAs and ITUs, which were mostly active against the wild type HIV-1 and retained the 'butterfly shape' active conformation observed in the TIBOs. However, the ITUs lack of *in vivo* activity was possibly ascribed to the oxidation of the core thiourea to a thiazole ring. Taking a leaf from the Cimetidine story, the bioisostere replacement of the thiourea moiety in ITU led to the serendipitous discovery of DATAs, which eventually featured a triazine core. Unfortunately, these potent inhibitors of wt HIV-1 were active against single-mutant strains of RT but ineffective against double-mutant strains of the enzyme.

This issue was addressed in the next series, the DAPYs, which were found to be potent inhibitors of HIV-1 RT wild type, single and double mutants. Further SAR studies finally culminated in TMC125 (Etravirine), which is superior to Nevirapine, Delavirdine and Efavirenz (which fail against mutant strains). Interestingly, molecular modelling showed that compounds such as TMC125 can adopt both a butterfly or horseshoe conformation, which enable them to be active against a variety of mutant strains. TMC125 is currently completing Phase II clinical trials for the potential treatment of naïve and NNRTI-resistant patients and has shown efficacy for both types of patients. Finally, a further analogue in this series, TMC278 (Rilpivirine), has proved an even more attractive compound with good microsomal stability and good half-life despite the presence of a Michael acceptor. It displays favourable pharmacokinetic properties and is a potent inhibitor of wild-type and NNRTI-resistant HIV-1. The compound is highly efficacious in monotherapy for seven days.

"The use of silicon as an isostere for carbon can lead to novel entities"

Exploiting the 'silicon switch' concept

Dr Graham Showell of Paradigm Therapeutics presented the approach adopted at his company to develop novel drugs by the clever use of the 'silicon switch' concept. Starting from a drug molecule that may suffer from a number of deficiencies, the company believes that the use of silicon as an isostere for carbon can lead to novel entities with desirable novel or improved features: 'novel compounds for known drug targets'. Indeed, the 'silicon benefits package' includes altered bond length and bond angles, increased acidity, increased lipophilicity, increased chemical stability and broader synthetic possibilities. This can improve potency, PK properties or even give new possibilities for delivery systems. The company has a strong proprietary chemistry with access to an impressive array of robust and high-yielding synthetic routes for the preparation of complex silicon-containing target molecules (eg Sila-Niguldipine). In addition, such 'silicon-switched' compounds have a strong IP position. Dr Showell then exemplified the concept with a number of fast follower projects in the main target families (kinase, protease, GPCR and nuclear receptor). For example, Haloperidol binds the D2 receptor with an IC_{50} = 5 nM, whilst a single silicon switch gives sila-haloperidol which already shows increased activity IC_{50} = 0.9 nM. Similarly, the issue of poor metabolic stability in niguldipine was addressed by a silicon switch and the resulting compound displayed increased activity. Bexarotene, a member of the retinoid family, used for treating T-cell lymphoma and lung cancer, gave sila-bexarotene with potential for an altered metabolic profile. Clinical trials for sila-bexarotene in humans remain to be carried out.

"Irreversible inhibitors are not desirable molecules for libraries"

Approaches to drug discovery

Dr Gilbert Rishton of California State University addressed a number of topical aspects of modern drug discovery. He examined the value of the therapeutic areas approach vs. target families approach and challenged that medicinal chemists should concentrate on the latter whilst leaving the former to pharmacologists.

Dr Rishton then described how the physicochemical properties of lead-likeness and the surprising differences

between those properties and the classical definitions of drug likeness are becoming apparent. The selection of non-covalent, high-affinity, reversibly binding ligands will provide a good lead compound for optimisation and drug development.

He then turned to de-selection of non-lead-like compounds, defining 'binders and bonders' as compounds that contain substructures that can irreversibly connect to a biochemical system. He said irreversible inhibitors, suicide inhibitors, and 'magic bullets' which were once fashionable are not desirable molecules for libraries. The 'magic bullet dogma' that prevailed after the discovery of aspirin and was held as an example of how drugs should be designed was subsequently proved wrong. The presence of the ester moiety made aspirin a pro-drug rather than a suicide inhibitor that acylated its target. Similarly natural products extracts should be appropriately pre-treated prior to inclusion in a compound collection because of their reactive functionalities.

Interestingly, the speaker suggested what chemical classes had a higher probability of being successful in his opinion (GPCRs > ion channels > proteases). He said medicinal chemists should develop the ability to distinguish between promising drug leads and the many useless false positives that can plague screening efforts. The evaluation of 'positives' should aim to select stable, non-covalent binders, ie ligands, and eliminate protein reactive compounds, ie reagents, from their initial lead selection.

Dr Rishton concluded that attention to small-molecule target class tractability, along with the application of lead-likeness criteria and drug-likeness criteria in the arenas of drug discovery and drug development, respectively, will assure the proper utilisation of biochemical tools and increase the chances of success in discovery programmes.

Electron fields and chemical diversity

Dr Tim Cheeseright presented an overview of Cresset Biomolecular Discovery's proprietary science, which describes molecules from the protein's viewpoint rather than their 'bare bones' structures (ie how they appear to other molecules). The company's eXtended Electron Distribution (XED) model allows the description of molecules in terms of surface properties or 'Fields'. Thus diverse structures such as peptides, steroids or organic compounds can encode the same binding environment and give similar patterns in their bound conformation. Since the XED is not dependent on the structure of the protein target (which can be unknown), the approach gives a powerful insight into the properties required for activity at a biological target.

The molecule 3D pattern is encoded with a 1D string (FieldPrint) and stored into a database as a distillate of important binding information regarding the molecule. Searching the database with the FieldPrint of an active molecule can retrieve molecules with a similar field pattern and a supposed similar biological activity to the initial search molecule. Hence the XED approach can be used to predict a molecule's biological properties for lead generation or for finding new leads from completely new chemical classes, as was shown in two worked examples.

In the thrombin area, starting from three diverse structures, FSN, PPACK and NAPAP, the programme first generated conformation populations for each active. Next, cross comparison of each pair of molecules showed that Fields can describe known diverse actives as similar. The concept was then extended to search for new, diverse actives and virtual screening from 600,000 compounds retrieved a number of known active inhibitors. A further validation was described in the CCK-2 Receptor area. Two active diverse molecules were used to create unique search field patterns. The initial search across 6,000 compounds was distilled to 1,000 compounds and then further reduced to 100 compounds following visual inspection. Eighty-eight of these were purchased and tested. Impressively, 27 had pKb > 5 (better than 10 micromolar) and 4 had pKb > 6 (better than 1 micromolar). More interestingly, 24 of the 27 had no structural similarity to known actives. All were in the MW range 350-600.

Finally, the speaker emphasised again how the approach can be extended to lead optimisation, hit rationalisation, hit identification or library design.

Synthesis and flash chromatography

Dr Farah Mavandadi of Biotage AB opened her presentation on integrating synthesis and flash chromatography with an historical coverage of the basis of microwave-assisted organic synthesis (MAOS) followed by an overview of the Biotage microwave equipment. The use of microwaves in shortening reaction times as well as speeding up the chemistry development process was aptly demonstrated in a number of worked examples. In addition to the better-known reactions, some interesting findings were reported in carbohydrate chemistry where intricate protection and deprotection reactions could be carried out under surprisingly mild conditions. A number of applications of MAOS to the synthesis of a number of drug molecules were presented with impressive typical total run times of 10-15 minutes.

It is, however, the combined use of MAOS, solid supported reagents as well as flash chromatography techniques (Biotage flash equipment was reviewed) that offers the best overall results, as was aptly demonstrated in a number of worked examples.

Conditions for the reductive amination of 'problem amines' were successfully developed using a combination of microwaves (decreased reaction time from 24 hr to 5 min), solid supported sodium cyanoborohydride and flash purification techniques to give an array of ten otherwise poorly accessible secondary and tertiary amines.

In another example, the four-step synthesis of tri-substituted triazines was developed from a literature route using MAOS, the safety-catch approach and Biotage flash chromatography equipment with an impressive 1-hour total run time (including purification) and gave products in 99% purity and 64% overall yield in four steps.

New potent Flurbiprofen analogues

Dr Ilaria Peretto of NiKem Research described research based on the clinical observation that there is a reduced prevalence of Alzheimer's Disease (AD) among non-steroidal anti-inflammatory drugs (NSAIDs) users. Some NSAIDs apparently selectively lower Ab42 levels. Ab42 is a highly insoluble, neurotoxic 42 amino acid peptide responsible for fibrils and plaque formation.

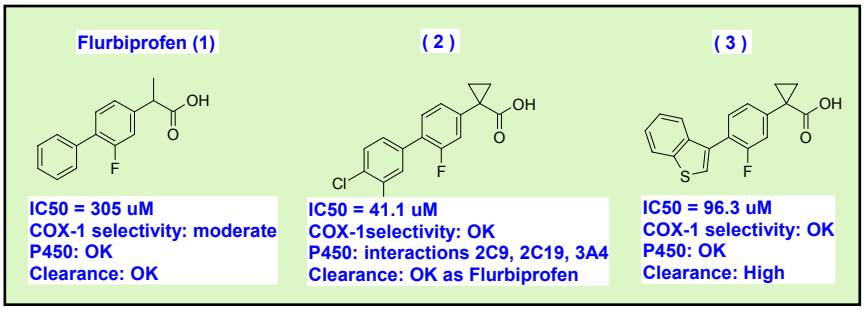

Fig 1. Screening of Flurbiprofen analogues as drug candidates for Alzheimer's Disease.

The aim of the work was to develop an NSAID that lowers Ab42 levels but is devoid of COX-1 activity, which is known to produce gastrointestinal side-effects.

Initial screening of commercial NSAIDs identified Flurbiprofen **(1)** (Fig 1) as a lead having the highest *in vivo* activity and the most consistent initial SAR.

Early SAR established that the biphenyl moiety as well as the propionic acid side-chain were key requirements for activity. Modulation of the terminal aromatic ring produced the best increases in potency. However, this first-generation compound lacked selectivity against COX-1 and had a poor P450 profile. COX-1 selectivity was addressed by introducing a secondary alpha substitution. However, attempts to address CYP 450 interaction by using a range of carboxylic acid bioisosteres were unsuccessful due to the stringent requirements for activity. The project now has a number of molecules that have good *in vivo* activity and selectivity but suffer either a poor P450 profile **(2)** or high clearance **(3)**.

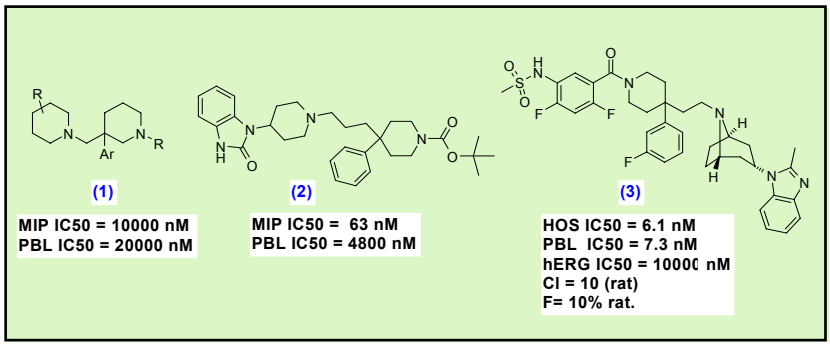

Fig 2. HTS of potential anti-HIV-1 agents.

Developing high-throughput chemistry procedures

The presentation given by Dr Marcus Bauser of Bayer HealthCare was a candid overview of the evolution of the company's high throughput chemistry procedures. The initial compound collection featured 300,000 compounds in 1996 (large libraries, mixtures and impure compounds) and has now evolved to a healthy 1.6 million compounds (boosted by acquisition via strategic alliances and the synthesis of focused libraries of pure compounds). Bayer has a state-of-the-art automated compound repository with a capacity for 3 million compounds. Aligned with the remainder of the industry, various Bayer sites contribute input for new libraries, the usual filters are applied and all structures chelated in an in-house database managed by proprietary computational design applications. Dr Bauser described a well-thought-out process, pre-empting the issues of frequent hitters as well as assay interference.

In practice, laboratory synthesis of smaller libraries is carried out using a range of technologies, and employing partially automated Bodhan liquid handlers as well as automated LC/MS sample purification techniques. The group has had significant impact in library generation with success stories in a number of areas including:

Automated reaction optimisation: Dr Bauser presented as an example the elaboration of an amino-quinazoline library aimed at improving ADME parameters for a medicinal chemistry project. Solvent, reaction times, and reagents were optimised in an automated fashion resulting in extensive time-saving and successful library synthesis.

Adenosine kinase inhibitor design: The initial 800-member library delivered a number of related 150-300 nM hits, which were worked into two subsequent 60- and 100-compound libraries to furnish a 10 nM drug-like compound.

P13K inhibitor with activity against vascular leakage

The talk by Dr Wolfgang Wrasidlo of TargeGen Inc outlined the programme followed by the company in developing the phosphoinositide-3 (PI3) kinase inhibitor TG100-115 for the treatment of acute myocardial infection. The programme was founded on the initial observation that vascular leakage at microcirculatory level, a kinase-mediated event, is a hallmark of ischemic disease.

The group carried out an initial PubMed natural compound search for structures with microvascular protective activity. The resulting hits were processed through an *in vivo* initial screen that visualised oedema reduction rather than anti-kinase activity. Analysis of actives identified a common structural motif, which led to the synthesis of a series of novel pteridines showing potent inhibition of vascular permeability in the *in vivo* screen. TargeGen thus developed extensive synthetic routes to pteridine analogues either by multicomponent core formation or by decoration of a central pteridine core. Most syntheses consist of high-yielding, one-step reactions using inexpensive materials to give novel patentable structures. Structure-activity profiling led to the selection of TG100-115 as the lead compound. In addition, pathway analysis identified TG100-115 as a potent PI3K inhibitor of high kinase selectivity.

Preclinical results show that TG100-115 prevents VEGF-induced phosphorylation of VE-cadherin, but does not affect ERK phosphorylation. TG100-115 also inhibits all three major isoforms of PI3K with IC_{50} values of 83, 235 and 1200 nM against gamma, delta and beta isoforms, respectively. The compound entered Phase II clinical trials in January of this year.

Developing an anti-HIV-1 agent

Dr Maosheng Duan of GlaxoSmithKline presented a vast amount of meticulous research work which included addressing the common issues of potency, DMPK and hERG. The initial HTS (Fig 2) gave a hit **(1)** with micromolar potency. In the cell assay the subsequent lead **(2)** was worked by a series of careful small focused library optimisations. Although early studies revealed that an unsubstituted endo-tropane benzimidazole had potent antiviral activity in HOS assay, tight SAR around the molecule only left the terminal western 'R substituent' available for designing out the hERG activity and little scope for improving the mediocre DMPK properties. A key modification was the introduction of a reverse sulfonamide moiety, which enabled the discovery of the final disclosed structure **(3)** with nanomolar activity (HOS), a clean hERG profile, acceptable clearance and bioavailability. *sp²*

FURTHER INFORMATION

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