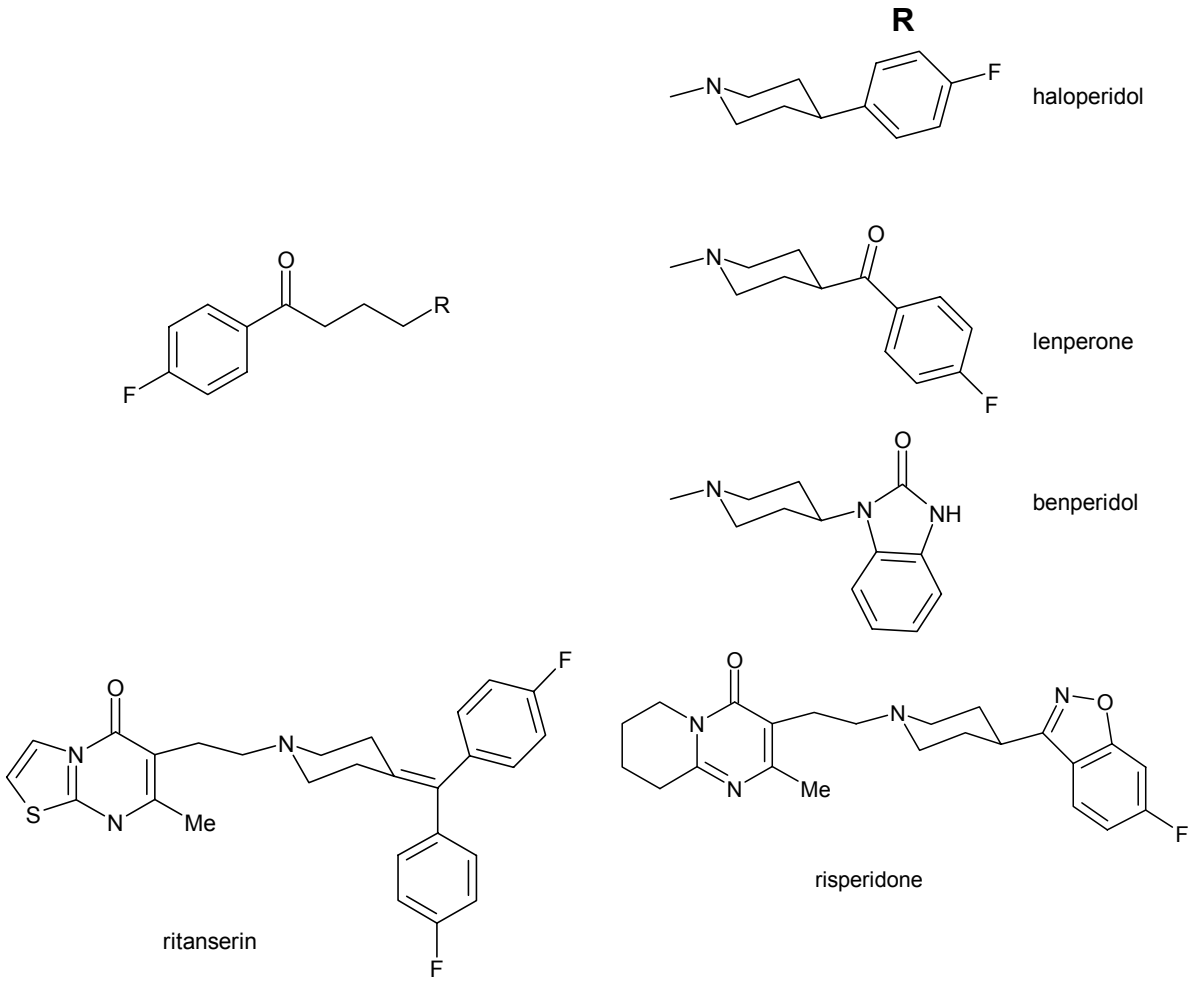
Case Histories in Drug Discovery and Design

On the 4th December 1997, the Society for Medicines Research organised a one-day symposium on "Case Histories in Drug Discovery and Design". The meeting incorporated the Society's Award for Drug Discovery, which this year was presented to Dr Sally Redshaw, Dr Ian Duncan and Dr Noel Roberts for their contributions to the discovery of the HIV proteinase inhibitor Saquinivir. Including the Award lecture, seven highly informative accounts of drug discovery and design were delivered by an international panel of speakers.

Anton Megens (Janssen, Belgium) described the historical background to the discovery of the antipsychotic agent Risperidone. Schizophrenia represents a major clinical and social problem, having a worldwide lifetime prevalence of approximately 1% and accounting for some 2.5% of total healthcare expenditures ($33bn in US in 1990). The vast majority of patients require long-term drug treatment and there is no cure for this serious and debilitating disease. Megens outlined the evolution of drug treatment for schizophrenia, starting from the observation in 1952 that chlorpromazine possessed "neuroleptic" activity. Chlorpromazine improved the positive psychotic symptoms (hallucinations, delusions), but produced Parkinsonian-like extrapyramidal side effects (EPS). Researchers at the time thought that this activity resulted from the serotonin antagonism of the compound. However, the introduction a few years later of haloperidol, a potent dopamine D_2 antagonist, as an antipsychotic agent and the results from many other observations such as amphetamine psychosis suggested that schizophrenia was related to an over-active dopaminergic system. In the 1970's pipamperone and clozapine were both shown to have an atypical clinical profile, resulting in a greatly reduced liability to produce the EPS of earlier drugs. These two agents shared the common property of combined dopamine and serotonin antagonism and it was subsequently demonstrated that the $5HT_2$ antagonist ritanserin, in combination with haloperidol, was effective in reducing EPS and produced some efficacy against the negative symptoms of schizophrenia (social withdrawal, lack of emotion). A medicinal chemistry programme, initially based on the neuroleptics lenperone and benperidol, ultimately led to the discovery of risperidone. Within a series of benzisoxazole derivatives, risperidone showed the desired combination of very potent serotonin ($5HT_2$) and potent dopamine (D_2) antagonism. In animals, risperidone differed from haloperidol in its ability to antagonise the stimulus effects of LSD and required high doses to induce catalepsy. In schizophrenic patients, risperidone has been shown to be effective against both positive and negative symptoms with a reduced EPS liability and, in many centres around the world, has become the first-line therapy for the treatment of this major CNS disorder.



Moving from the CNS to the CVS, the meeting heard a presentation from **John Maraganore** (ex Biogen, now Millennium Biotherapeutics, US) on the direct thrombin inhibitor, Hirulog™ (bivalirudin). The 20 amino acid peptide drug was rationally derived from hirudin, a 65 amino acid natural product from the medicinal leech, and designed to bind both to the anion-binding site and the catalytic site on thrombin. It inhibits thrombin selectively with a K_i = 2nM. The unnatural configuration of D-Phe(1) was incorporated based on prior knowledge of inhibitors, and the overall binding mode has been confirmed by an X-ray crystallographic structure of thrombin with the bound inhibitor. Until recently the synthesis was a 36 step procedure but now manufacture is by a semi-synthetic recombinant DNA process to give an 18mer, with the remaining amino acids added synthetically. Hirulog™ has undergone extensive clinical trials and has now been licenced from Biogen to The Medicines Company. It is being developed as a safer more effective anticoagulant

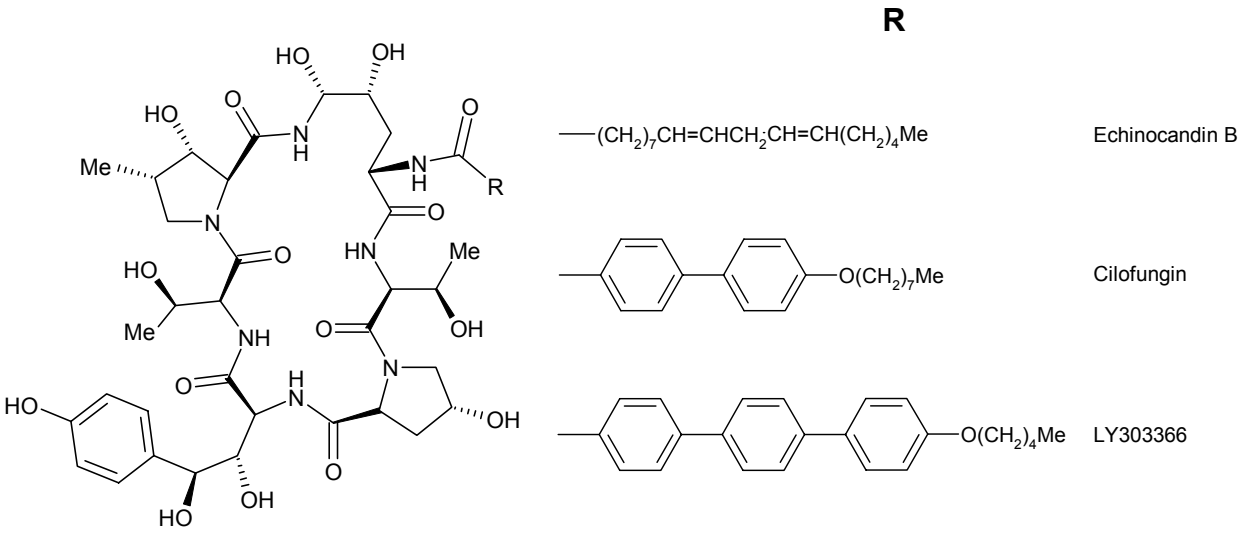
than heparin and has been studied in numerous settings: the prevention of deep vein thrombosis, treatment of unstable angina, treatment of acute myocardial infarction during thrombolysis and prevention of acute complications of percutaneous transluminal coronary angioplasty (PTCA). It is proving to be superior to heparin in several respects: more potent and effective, has a direct thrombin inhibition, effective in the presence of existing thrombosis, no detrimental effects on platelets, and lower systemic coagulation. It is expected to be launched in 1999 for angioplasty. A major mortality trial in AMI (17000 patients, HERO II trial) is under design.

D-Phe-Pro-Arg-Pro-Gly-Gly-Gly-Gly-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu

Hirulog™

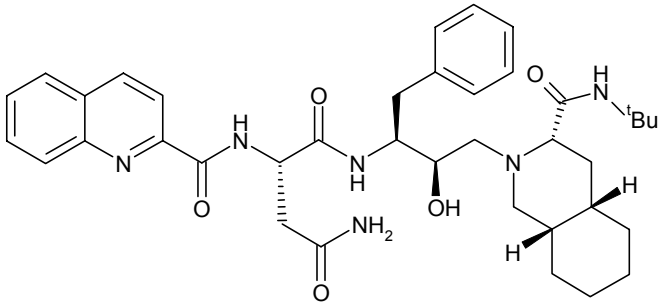
Stephen Hitchcock (Eli Lilly & Co., Indianapolis) described the discovery of the antifungal Echinocandin derivative LY303336. Over the last three decades there has been a dramatic increase in the incidence of fungal infections. This is due to the advent of AIDS and other immunosuppressing conditions, together with other factors such as the demographic shift towards an older and more infection-prone population. Until recently, the treatment of serious fungal infections relied primarily on amphotericin B, but the use of this drug is limited by its toxicity. The newer triazole derivatives, such as fluconazole, are effective and widely used. However, these agents are fungistatic and there are some concerns that they may lead to the development of resistance. Natural products have provided several novel leads as antifungals and of these, the echinocandin lipopeptides are of considerable interest due to their excellent fungicidal properties and low toxicity. Their mode of action uniquely targets fungal cell wall synthesis by inhibiting the enzyme β-1,3-glucan synthase, preventing the production of β-1,3-*D*-glucan (an essential cell wall component). Echinocandin B (ECB) consists of a cyclic hexapeptide whose N-terminus is acylated with linoleic acid. Fermentation of ECB with *Actinoplanes utabensis* produced the core cyclic peptide and allowed the synthesis of a wide range of replacements for the linoleoyl side-chain. From this work a series of alkoxybenzoyl derivatives was prepared, leading to the discovery of cilofungin. Clinical studies confirmed that cilofungin had excellent antifungal activity and low toxicity. Unfortunately, this compound suffered from low aqueous solubility and a short terminal half-life. A second generation chemical programme was therefore initiated, with the aim of improving the solubility and pharmacokinetic profile. It was found that the shape of the lipophilic side chain was important, with linear, flexible chains required for optimal antifungal activity. From these studies, the alkoxypolyaryl derivative LY303366 emerged. This analogue was shown to be more effective than cilofungin in experimental C. albicans infection models and had a broader spectrum of activity. Further studies showed LY303366 to

be orally effective and formulation of the compound overcame the problems with low solubility. LY303366 is currently in advanced Phase II trials.



On behalf of the award-winning team, **Noel Roberts** (Roche, UK) delivered a fascinating account of the strategy and drug design which contributed to the discovery of the HIV proteinase inhibitor Saquinavir. The research programme at Roche was initiated in the mid-1980's. At this time only very provisional information was available concerning the classification and structure of the HIV proteinase enzyme and the importance of the enzyme in the virus life cycle. HIV proteinase is unusual in its ability to cleave the Phe-Pro and Tyr-Pro sequences found in *gag* and *gag-pol* gene products. Since mammalian endopeptidases are not able to hydrolyse the amide bonds of Pro residues, the team reasoned that this could provide the basis for the rational design of selective inhibitors. A series of compounds based on the *pol* fragment Leu^{165}-Ile^{169}, containing the transition-state mimetic Pheψ[CH(OH)CH_2N]Pro in place of the Phe^{167}-Pro^{168} scissile bond were prepared in order to determine the minimum sequence required for potent inhibition of the enzyme. This led to the identification of Z.Asn.Pheψ[CH(OH)CH_2N]Pro.O^{t}Bu as an inhibitor with an IC_{50} of 140 nM against HIV-1. Subsequent exploration of the optimal requirements at each of the side chain positions resulted in the synthesis of Ro 31-8959 (saquinavir). This compound is an extremely potent (Ki <0.1 nM) and specific inhibitor of HIV proteinase (having no significant inhibitory effect on the human aspartic proteinases at 100,00 times this Ki concentration). Antiviral activity in the low nanomolar range, combined with oral bioavailability and a clean safety profile resulted in the

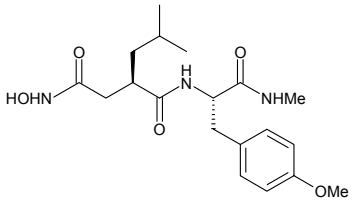
development of saquinavir as the first HIV proteinase inhibitor to become available as a marketed drug (Invirase®). Invirase® has demonstrated significant clinical benefit in terms of reducing by 50-70% the risk of AIDS-defining events and death in HIV infected patients. The very good tolerance seen with Invirase® has allowed a reformulation of saquinavir as Fortovase™ which delivers a higher drug exposure to the patient. Clinical results have demonstrated that 80% of patients taking Fortovase™ plus two nucleoside RT inhibitors have viral levels below detection after 16 weeks of treatment.



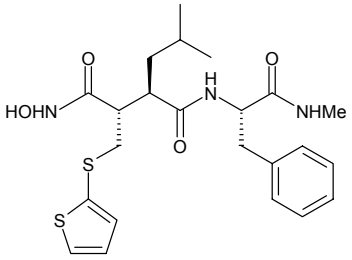
Ro 31-8959 (saquinavir)

The meeting continued with an antiinfective theme with two presentations on different mechanism of action anticancer compounds. **Mike Crimmin** (British Biotech Pharmaceuticals, UK) described the rationale behind marimastat (BB-2516), a matrix metalloproteinase (MMP) inhibitor, and its use in cancer therapy. MMPs, for example collagenases, stromelysins, gelatinases, possess the ability to degrade the components of the extracellular matrix. MMP activity is closely regulated by several mechanisms and when an imbalance occurs, cancer and other pathological conditions such as arthritis can arise. High levels of MMPs are expressed in a wide variety of cancer cells (for example, colorectal, lung, oesophageal, breast, ovarian, gastric and prostate) and are implicated in various stages of the aetiology: metastasis, local invasion and angiogenesis. Design of MMP inhibitors therefore, has been an area of intense activity in the pharmaceutical industry. This drug design programme was based on knowledge of the collagenase cleavage site and molecules incorporated features to allow them to co-ordinate with the active site zinc atom - a hydroxamic acid group, and sidechains that allowed interaction with the enzyme sub-sites, left and/or right of the active site. In this instance, sidechain interactions were found to subsites to the right of the active site i.e. the S1' and S2' pockets. An early lead, SC 44463, had potency in the 10nM range against collagenase. Introduction of substituents alpha to the hydroxamic acid led to batimastat (BB-94) which proved to be a low nM agent against a range of

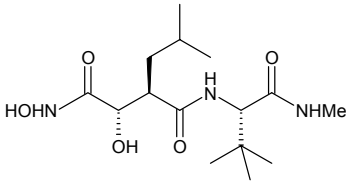
MMPs. *In vivo* studies with batimastat led to vastly improved survival in rats bearing breast tumours,. The compound, however, had very low aqueous solubility (0.003mg/ml) and no oral bioavailabilty. Further modifications led to marimastat which is much more soluble (7.7mg/ml) than earlier compounds. Anticancer activity was demonstrated in a mammary carcinoma model, in which marimastat caused a reduction in tumour burden at 30mg/kg po. In Phase I studies, the compound has a $t_{1/2}$ in human volunteers of 12-13h, with C_{max} around 2h. In Phase II studies, reduction in the rate of rise of cancer antigen was measured (ovarian, colorectal, prostatic and pancreatic cancers). Significant decreases in the rate of rise of
cancer antigens was observed in the treated groups (5mg to 50 mg bid) when compared with the rate of rise prior to treatment. Phase III trials (Europe and North America) have commenced in pancreatic cancer, small cell lung cancer, malignant brain cancer, stomach cancer and ovarian cancer, whilst pilot studies in breast and colorectal cancer are underway.



SC 44463



Batimastat

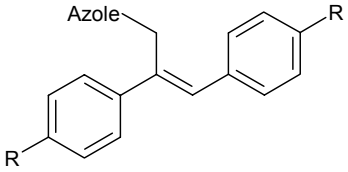


Marimastat

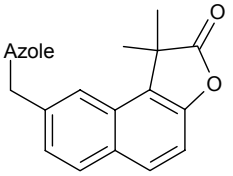
The second anticancer drug case history was given by **Tom Boyle** (Zeneca, UK) who outlined the development of the selective aromatase inhibitor Arimidex™ for the treatment of breast cancer. This is the commonest cancer in women and despite advances in treatments, world-wide mortality from the disease is increasing. In post-menopausal women, one of the most effective treatments of breast cancer has been to deprive the tumour of one of its primary mitogens - oestrogens. The Zeneca strategy was to prevent oestrogen synthesis by inhibition of aromatase, the ultimate and unique enzyme that converts androgens, such as testosterone, to oestradiol. For efficacy studies, acute inhibition assays were carried out in rodents (aromatase only present in brain and ovaries), and chronic inhibition of peripheral aromatase was measured in macaque monkeys. Aromatase is a P450 enzyme with single gene origin, regardless of tissue. To help the drug design, it was possible to use the structure of the P450(camphor) as a basis for a homology model with a steroid substrate docked in. Initial lead compounds were azoles incorporated onto non-steroidal oestrogen-like scaffolds (for example, stilbenes, naphthol lactones). The binding hypothesis was that

the azole mimicked oxygen in the enzyme, by binding to the haem, thus preventing hydroxylation. Imidazoles were found to be more potent than triazoles *in vitro*, but the potency order was reversed *in vivo* owing to metabolic stability. Chance synthesis of a nitrile intermediate needed for the naphthalene analogues and further elaboration eventually led to the potent and selective aromatase inhibitor, Arimidex™. The compound has an IC_{50} 4ng/ml and an ED_{50} 0.015mg/kg. Against human placental aromatase *in vitro* it is a 15nM inhibitor.

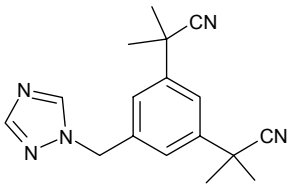
In the acute ovulation assay, Arimidex™ has an ED_{100} 0.1mg/kg po. It is very selective with no effects at 30-200X MED versus other P450 enzymes. In DMPK studies, the $t_{1/2}$ for rat is 7h, dog 9-16h, monkey ~8h and man 48h. Primary metabolism is by N-dealkylation of the azole to give a benzoic derivative. Just 1mg/day gives >95% aromatase inhibition of total body aromatase in post-menopausal breast cancer women.



stilbenes



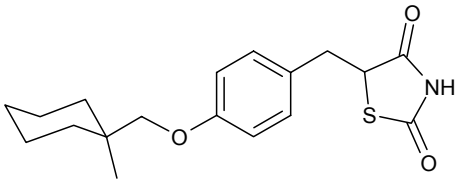
naphthol lactones



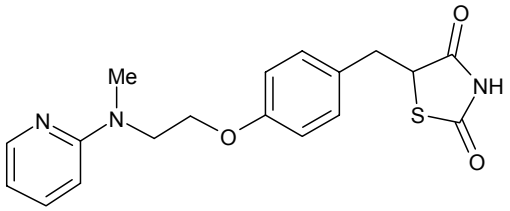
Arimidex™

The meeting finished with a concise account of the discovery of an antidiabetic agent, the PPAR-gamma agonist, rosiglitazone, described by **Steve Smith** (SmithKline Beecham, UK). This was an example of drug discovery in which the compounds have been identified from *in vivo* studies using animal models of the disease state, with subsequent research identifying the molecular basis for the activity; hence, from screen to gene. Type II diabetes, non-insulin dependent diabetes (NIDDM) accounts for 90% of all diabetics. The disease is characterised by a relative insulin deficiency, glucose utilisation defects, and progressive failure of pancreatic beta-cells. Current therapy is complex and comprises combinations of diet, oral agents and insulin. Clinical diagnosis is generally 9-12 years after the development of the disease and affects >120 million people world-wide. There are many predisposing factors: age (>40), polygenic genetic factors, excess calorie and saturated fat intake, and obesity. The financial costs are >$100 billion (USA) and >10% total NHS spend (UK). The lead for the drug discovery programme was the thiazolidinedione, ciglitazone, found in the early 1980s by Takeda, when searching for fibrate-like hypolipidaemic agents. Long term studies with ciglitazone lowered blood glucose and plasma insulin in rats, normalised hyperglycaemia and improved tissue insulin sensitivity in the obese mouse, and indicated a slow onset of action (>12 days); but the antidiabetic activity was weak. Rosiglitazone (BRL 49653) emerged from an SAR programme on ciglitazone in which

the lipophilic cyclohexyl group was replaced by aromatic and polar groups. Thus, replacement with a phenylurea and conformationally restrained derivatives, such as benzoxazoles, gave substantially more potent analogues. An aminopyridine derivative, rosiglitazone, was chosen for development. In biological studies, it reduces hyperinsulinemia, reduces triglycerides and free fatty acids, enhances insulin stimulated glucose uptake into adipocytes (via glucose transporter GLUT-4) in the obese mouse, and increases total GLUT-4 at the cell surface through stimulated translocation of the protein from an internally sequestered state. *In vivo*, rosiglitazone is a potent and selective, orally active antihyperglycaemic agent in rodent models of Type II diabetes and, unlike sulphonylureas, there is no hypoglycaemia and no increase in insulin secretion. The molecular target of rosiglitazone now known to be a nuclear hormone receptor, the peroxisome proliferator activated receptor gamma subtype - PPAR-gamma - at which rosiglitazone acts as an agonist. The receptor subtype is predominant in adipose tissue and is present at far greater levels than in liver and skeletal muscle. Activation of the receptor leads to enhanced expression of adipocyte-specific genes, in turn causing differentiation of pre-adipocytes into adipocytes, and enhanced expression of GLUT-4. In clinical studies, in a Phase II dose-ranging trial in diabetic patients (a 12 week treatment period), using fasting blood glucose concentration as an endpoint, doses of 1 and 2mg bid gave a highly significant reductions in fasting hyperglycaemia. The compound is currently in Phase III clinical development.



ciglitazone



rosiglitazone