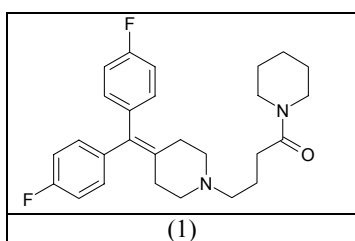


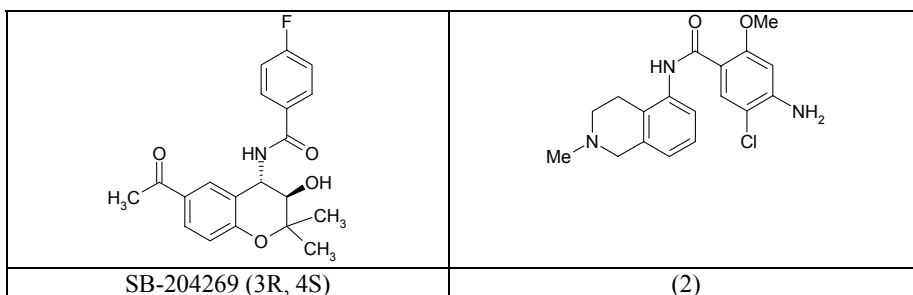
Trends in Medicinal Chemistry, Report

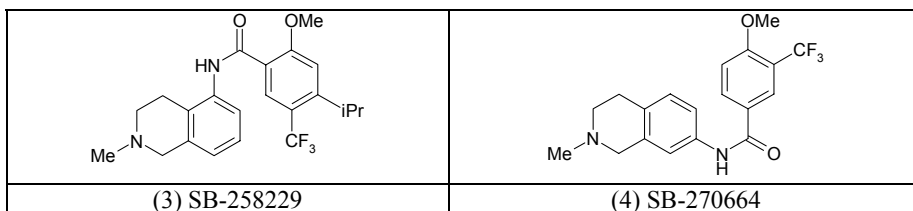
The Society for Medicines Research held a highly popular meeting attended by an audience of 140 entitled Trends in Medicinal Chemistry on 3 December, 1998 at the National Heart and Lung Institute, London. The meeting is intended to alert researchers about compounds in early development, or new discoveries likely to lead to new approaches to diseases treatment in the near future.

The symposium opened with an exposé by Dr David Jaap of Organon's work in the design of novel atypical anti-psychotics. For many years the prototypical compound in this area has been clozapine, which despite its advantages in lacking many of the extrapyramidal effects of typical antipsychotics, is substantially limited by the side effect of agranulocytosis, which affects about 2% of patients. The team aimed to produce D₂ antagonists which lacked cataleptic effects at D₂ antagonistic doses. They developed a D₂ pharmacophore model, and screened their database of potentially active compounds. Initial leads were poorly active *in vivo*, and this element was tackled by the incorporation of two 4-fluoro groups in (1). ORG 23366 showed a pK_i of 7.1 and activity in the apomorphine climbing model at 0.5 mg/kg (s.c.) or 2.8 mg/kg (p.o.).

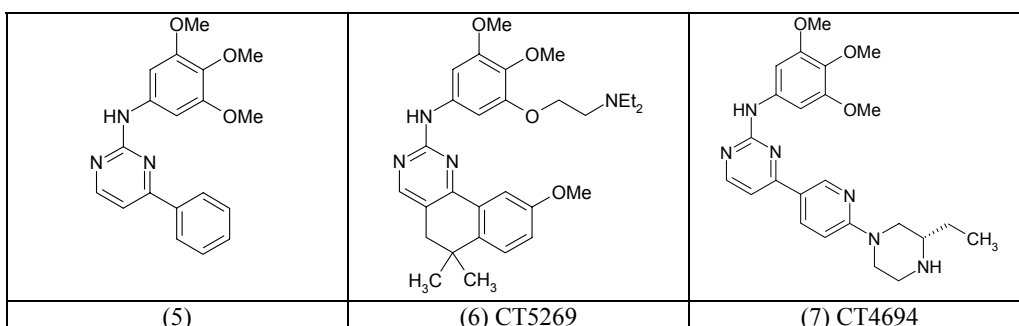


Maintaining the theme of CNS therapy, Dr John Harling (SmithKline Beecham) presented the following story to the discovery of SB-204269 (first revealed at the SMR's Epilepsy meeting in April 1996, and now in Phase II), a new anticonvulsant acting at a novel binding site in the brain. This compound initially came out of a project to discover neuronally active ATP-sensitive K⁺ channel openers based around levocromakalim, with increased lipophilicity for brain penetration. Further work made it clear that the enantiomer lacking channel opening activity was responsible for the anti-convulsant effects *in vivo*. High throughput screening (HTS) using a [³H]-SB-204269 binding assay led to the discovery of a novel series of 1,2,3,4-tetrahydroquinolinyl benzamides that also have anticonvulsant activity. Initial investigations into SAR on the lead compound (2) in this novel series, were undertaken using parallel synthesis methods, facilitated by the presence of the amide linkage. The need for a tetrahydroisoquinoline heterocycle was established and the location of the amide was shown to be optimal at positions 5 and 7. The order of potency for isoquinoline nitrogen substituent was Me, H > larger alkyl. In the 5-series, preparation of a range of substituted benzamides defined the need for an ortho methoxy group (which forms an intramolecular H-bond), a bulky lipophilic substituent para to the amide, and a halogen or CF₃ para to the methoxy group, e.g. SB-258229 (3). Despite high affinity in the binding assay and good *in vivo* activity in a rat MEST (maximal electroshock threshold) test, the compounds were not sufficiently selective over the 5-HT_{2C} receptor. This latter activity was subsequently attributed to the ortho methoxy group. Compounds of the 7-series without an ortho methoxy group, e.g. SB-270664 (4), were shown to have the required selectivity (≥100 fold over 5-HT_{2C} receptors) and oral activity in the MEST test at 2mg/kg po.

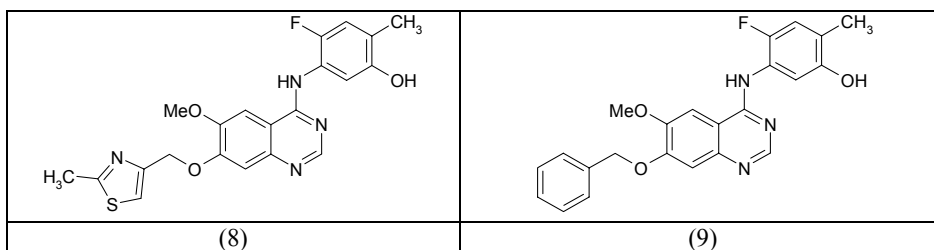




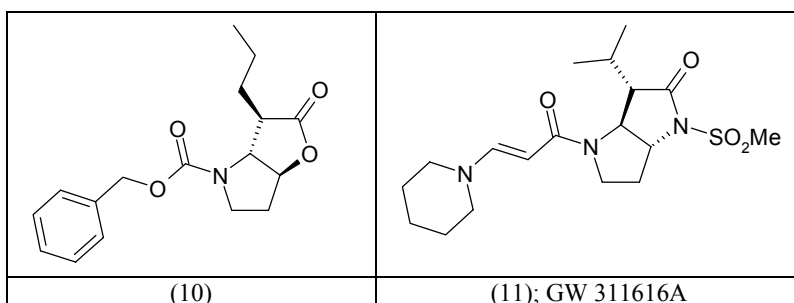
The theme of the meeting for the next two talks turned to tyrosine kinases. Dr Mark Batchelor presented the work at Celltech on inhibitors of these enzymes as potential immunosuppressant compounds. Activation of T lymphocytes proceeds via T-cell receptors (TCR) and is mediated by a tyrosine phosphorylation process. ZAP70 and p56lck are two cytoplasmic phosphotyrosine kinases (RTK) that are activated upon TCR ligation. This couples TCR to downstream signalling and leads to the activation of several pathways, for example, PLC γ 1, Rho/Rac and Ras/Raf, which ultimately affect gene transcription and cytokine production. Low micromolar inhibitors of p56lck (src family of RTK) were identified by HTS, the most promising being a set of 2-aminopyrimidine derivatives, of which (5) was chosen for lead optimisation. Low nanomolar potency inhibitors, such as CT5269 (6) (IC₅₀ 1.1nM), were obtained by constraining the torsion angle of the aromatic rings and introducing substituents to interact with key residues identified in the crystal structure of the ATP catalytic binding domain of the enzyme. Whilst having selectivity over other families of RTKs, this was not so within the src family (e.g. fyn, lyn and yes). The compound did however have activity in functional cellular assays which monitored calcium flux and IL-2 production. Potent ZAP70 inhibitors, such as CT4694 (7) (IC₅₀ 7.8nM), were also derived from 2-aminopyrimidines, and shown to have selectivity over lck.



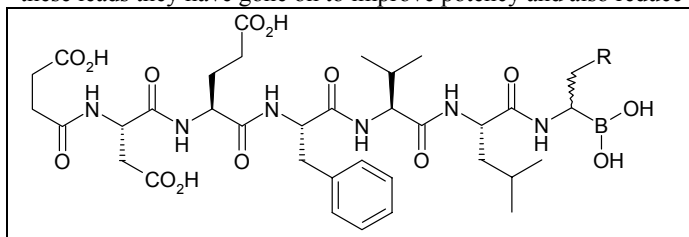
The role of tyrosine kinases in cancer, specifically in relation to vascular endothelial growth factor (VEGF) was explained by Dr Andrew Thomas (Zeneca). VEGF is a mitogen for vascular endothelial cells and has a specific and direct action on these cells. Its major roles are to stimulate angiogenesis and increase vascular permeability, processes involved in growth and progression of tumours, psoriasis, diabetic complications and rheumatoid arthritis. At the molecular level, VEGF is a ligand for two VEGF receptor tyrosine kinases, Flt and KDR, which undergo receptor dimerisation and initiate intracellular protein phosphorylation. The lead compound for the design of VEGF inhibitors was from series of anilino-quinazolines made initially as EGF RTK inhibitors. Initial SAR was performed using robotic array synthesis and led to compounds with low nanomolar inhibitory potency. Further refinements gave compounds such as (8), (9) with selectivity over EGFR in kinase inhibition assays, and similar potency in a whole cell assay, VEGF-driven proliferation of human umbilical vein vascular endothelial cells (HUVEC). Selected compounds also showed oral activity in a uterine oedema model in which VEGF has been shown to be involved.



The afternoon session began with a polished presentation from Dr Mike Dowle (Glaxo Wellcome) on the story of the discovery of novel inhibitors of elastase for the treatment of chronic obstructive pulmonary disease. COPD is a complicated condition involving a combination of bronchoconstriction and emphysema, with excessive mucus production. Most incidence (80-90%) is found in smokers, affecting about 10-15% of this group. The disease is very poorly treated at present, but there is a body of opinion suggesting that elastase, an enzyme found in neutrophils is a good target for inhibition. Elastase is a 29kD single chain serine protease present in the granules of neutrophils, which are recruited into the lung in association with COPD. Its pathological role is three-fold: it plays a part in lung damage through causing connective tissue damage, inhibits mucociliary clearance and has inflammatory effects. GW's first lead was a triterpene from the wild sage *Cantana camara*. However, the compound also affected thrombin and trypsin, other serine proteases. The active pharmacophore of the molecule was defined as a 5,5-fused ring system, and this simplified molecule (10) was more selective for elastase over the other enzymes. Optimisation of the molecule focussed on improving its short half life, and activity was determined in a hamster model of lung inflammation following IL-8 administration and bronchoalveolar lavage with counting of neutrophils. The optimised compound, GW 311616A (11), has an IC_{50} of about 100nM, but a long duration of action due to absorption into bone marrow. An x-ray structure of the compound in association with porcine pancreatic elastase showed the orientation of the molecule in the active site, and binding of the acrylamide moiety to Val216. The isopropyl group fits into a lipophilic pocket. The lactam moiety in the structure is cleaved by the Ser195 of elastase, and the carbonyl group is activated by the attachment of the sulfonyl group. GW 311616A is a potent, selective, low MW, irreversible or slowly reversible elastase inhibitor based on a novel template. It is orally bioavailable and currently in Phase II clinical trials.

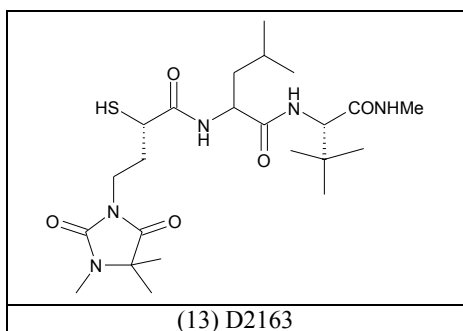


Serine proteases continued as the topic for the next talk, given by Dr Tony Raynham (Roche UK). Hepatitis C virus is an increasingly recognised disease, currently thought to affect 2% of the population, but prevalence rising due to more routine blood screening. The disease is commonly transmitted through blood transfusion, or needle sharing among drug addicts. Although in serious cases it can lead to severe chronic hepatitis, cirrhosis and hepatocarcinoma, in about 50% of cases the disease is mild enough to be asymptomatic. It is presently unclear what proportion of these will eventually progress to the more severe forms of the disease, and over what time frame. The HCV genome translates into a single polyprotein of 3020 amino acids, which is processed into at least ten cleavage products by a series of host and viral proteases. One of these proteins, NS3, has an N-terminal portion that is a serine protease, which is responsible for the cleavage at the NS3-4A, NS4A-4B, NS4B-5A and NS5A-5B sites. The target was to identify a substrate-based inhibitor of the NS3-4A protease. Roche have a successful history in enzyme inhibitor design, as evidenced by the discovery of the HIV protease inhibitor saquinavir for the treatment of AIDS. They used similar principles of active site analysis and structure-based inhibitor design, sequential modification of the P_1 to P_6 sites, and incorporation of an electrophilic moiety at P_1' to trap the serine group of the enzyme's active site. With this approach, they arrived at a series of aldehydes and boronic acids of which Ro 32-6167 and 32-6168 (12) were the most promising, with enzyme affinities of around 30-40 nM. From these leads they have gone on to improve potency and also reduce molecular weight.



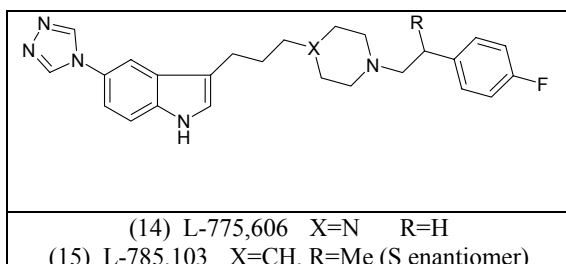
(12) R = Me, Et

Another group of enzymes which have attracted a large amount of attention over the years for their potential role in disease is the matrix metalloproteinases. The MMPs are part of a larger group of metalloproteinases which include angiotensin converting enzyme (ACE). They can be divided into 3 types, the collagenases, gelatinases and the stromelysins. Each is typified by a catalytic domain, a hinge and a C-terminal domain. Each MMP can cleave a number of substrates, and each substrate can be cleaved by a number of enzymes. This suggests that a degree of redundancy is evident in the system, and when coupled with the finding that a number of MMPs are released by most tumours, supports the theory that a degree of breadth in inhibition across a number of enzymes is preferable for therapeutic efficacy. MMPs have been proposed for the treatment of several diseases including inflammation, but Dr John Montana's presentation (Chiroscience) focussed on their value as anti-cancers. MMPs are implicated in a number of stages in the development of a tumour: from the breakdown of the extracellular matrix and the invasion of host tissue; the shedding of cells from the primary in order to produce secondary tumours; and the generation of new blood supplies to support the growth of a tumour. However, the first generation of MMPs such as British Biotechnology's marimastat and Agouron's AG-3440 were associated with a number of side effects including joint pain and tendonitis. These compounds affect other enzymes such as TNF-alpha converting enzyme (TACE) and other events involving the so-called 'shedases'. Chiroscience's approach was to aim for an orally bioavailable, non-specific MMPI, but without the side effects of tendonitis associated with the earlier compounds. Early on, the group identified the thioamide group as a replacement for the hydroxamic acid present in many first generation compounds. This functional group also had a much clearer patent position, less fettered with prior art, but more importantly delivers greater solubility, a severe deficit of the hydroxamic acids. This symposium occasioned the first public disclosure of the lead compound, D-2163 (13), a broad spectrum MMPI with low nM potency against a number of enzymes but which does not affect the shedases or other metalloproteinases. It has oral activity at 30 mg/kg in a rat model of cancer, and in pharmacokinetic experiments is shown to be much better absorbed than marimastat. Tolerance studies in marmosets (30 or 100 mg/kg once a day for 3 months) showed no effects on gross movement, and knee joint histopathology showed no difference from control. The compound was progressed into Phase I human volunteer trials where a single dose escalating study (25 – 900 mg) showed dose-proportionality in plasma concentrations up to 600 mg, with peak levels much above the enzyme IC₅₀, extending beyond 12 hours. At 600 mg, peak levels of 10ug/ml were noted. D-2163 is progressing into proof of principle studies in humans.

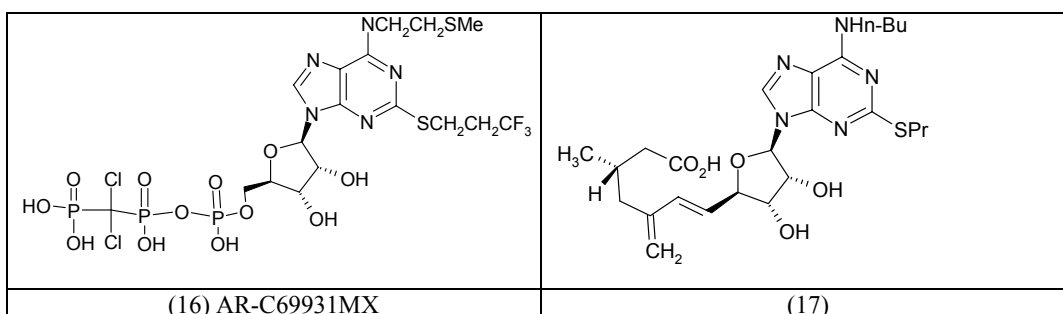


Dr Tammy Ladduwahetty (Merck UK) started her presentation by noting that 1998 was a landmark year for Terlings Park, as it has seen the launch of the first drug to emanate from that particular institution. Maxalt (MK-462, rizatriptan) is a dual 5-HT_{1D/1B} agonist which is more rapid in onset than sumatriptan and less active at other 5-HT receptors. In looking for a back-up, Merck wanted greater selectivity against 5-HT_{1D} receptors. This is because 5-HT_{1B} receptors are involved in the vasoconstrictor action of this class of anti-migraine compounds (which restores blood flow to the brain by shutting off the cerebral arteriovenous shunt), whereas both 5-HT_{1B} and 5-HT_{1D} receptors are involved in the neurogenic aspects of the migraine pain. Vasoconstriction is also likely to be responsible for the cardiac symptoms, and chest problems associated with sumatriptan, and it was thought that this could be avoided in a 5-HT_{1D}-selective compound. They sought a selectivity of greater than 50-fold, bioavailability of greater than 10% and CNS penetration. They started with structural elements from ketanserin, which has a 1D/1B selectivity of 25. Early leads such as L-772,405 and L-760,790 showed good potency and selectivity but poor bioavailability in rats. Further modifications led to L-775,606 (14), with a 1D/1B selectivity of 125, a 1D/1A selectivity of 55 and a

binding K_i of 0.6nM against 5-HT_{1D} receptors. The compound is a full agonist against 5-HT_{1D} receptors, and has a bioavailability of 27% and half life of 1.9 h in rats. In further improvements, the Merck team were able to increase the bioavailability, against problems of potency. In L-785,103 (15), the group had a full 5-HT_{1D} agonist (K_i 0.9 nM), $t_{1/2}$ 3.1 h, and excellent 1D/1B selectivity. However, wider selectivity in this compound against D2, M2 and other receptors was troublesome, and pharmacological evaluation *in vivo* showed adverse cardiovascular effects. This remains a stumbling block, but one that Merck are optimistic they can overcome.

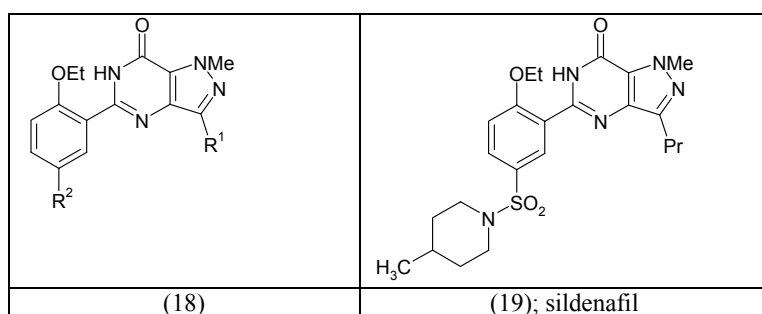


Over the years, adenosine receptors have received much attention as therapeutic targets, but the widespread distribution of the P1 purinergic subtype has limited the development of agents selective for this receptor class. Now the purinergic field has opened considerably with the revelation of receptors for ATP, namely P2T, P2X, P2Y and P2Z subtypes. Astra have been particularly active in this field, and Dr Tony Ingall presented their success in the area of P2T inhibitors for the treatment of thrombosis. Both ATP and ADP are ligands for this receptor, as weak antagonists and agonists respectively. The Astra team's initial approach in ATP analogues was to stabilise the terminal phosphate linkage, since they wished to avoid the problem of agonism once the terminal phosphate had been lost. Using a dichloromethylene linkage in place of oxygen achieved both enzymatic stability and retained the ionisation behaviour that was important for activity. They looked at modifications in the 2-position of the purine ring, and identified an early lead, AR-C69931MX (16) that showed a pIC_{50} of 9.4 and was selective over P2X and P2Y receptors. To avoid the problems associated with long-term increased bleeding time, they sought an ultra-short acting compound. With AR-C69931MX they showed in humans that the compound was safe and well-tolerated, had a $t_{1/2}$ of 2 minutes and when administered intravenously, had a rapid onset and offset in terms of platelet aggregation inhibition. Moreover, experiments in dogs (femoral artery flow reduction model) revealed that there was a much greater separation between platelet inhibition and bleeding time than with GpIIa/IIIb inhibitors. These observations were paralleled in humans, with no diminution of platelet inhibitory effect in patients treated with glycerine trinitrate, heparin or aspirin. These studies also established complete recovery of platelet function 20 minutes after stopping the infusion. Finally, the team has now moved on to compounds lacking the phosphate group, and have identified (17) as a potential development compound. It has a pIC_{50} of 9.5 and a comparable pharmacokinetic profile to AR-C69931MX.



The final talk of the day was reserved for the only marketed compound of those presented, and one which has been the topic of substantial press coverage, both scientific and non-scientific. Viagra (sildenafil) is a landmark in the treatment of penile erectile dysfunction (ED), and its discovery makes an interesting story. The Pfizer project that led to sildenafil was initially directed towards new anti-angina compounds based on the inhibition of phosphodiesterase type 5. PDE5 is found in smooth muscle and platelets, and inhibitors potentiate nitric oxide (NO) production, consequent vasodilatation,

and anti-platelet effects. Selectivity over type 3 was sought because of the adverse cardiovascular effects known to be associated with such compounds. Starting from zaprinast, the group looked at variation in the R1 and R2 positions of (18). The former was lengthened to a propyl group; a range of substituents were found to be acceptable for R², but sulfonamides were preferred, partly because of their low logD value. Ultimately, the piperidiny sulfonamide group was selected, and sildenafil (19) identified, a potent (IC₅₀ 3.6 nM) PDE5 inhibitor selective over PDE1 and PDE3 subtypes. The compound is 41% bioavailable and has a half life of 4 h in humans. Human trials were initiated in July 1991, and despite the failure to produce the desired cardiovascular effects, the effect on penile erections were noted in 10-day tolerance studies in early 1992. Serendipity played a part in the discovery of sildenafil's final therapeutic use, but there is a clear message of the benefit of good clinical design and observation. The role of NO in erectile dysfunction was also established around this time, as an agent that is released by NANC nerves and which stimulates guanylate cyclase to produce cGMP, the ultimate signal responsible for regulation of muscle tone in the penis. Dysfunction can be a vascular or a nervous disorder, but NO is involved in either pathology. It is estimated that there are 30 million sufferers of ED in the US, and the disorder afflicts 50% of all 40-70 year olds. Proof of the value of sildenafil in ED was established in 1994, and subsequent trials showed both the outstanding benefit and enormous popularity of the treatment.



Summary

This was a highly popular and well-run meeting. The talks were consistently of a very high quality, and a broad range of subjects covered. In all 10 pharmaceutical companies were represented, and presentations of potential therapies for CNS disorders, through immune disease and cancer to cardiovascular diseases were covered.