Trends in Medicinal Chemistry

by SMR Committee

The Society for Medicines Research held a one-day Trends in Medicinal Chemistry meeting on 30 November 2000, fittingly at the Research and Development site of Glaxo Wellcome in Stevenage. As in previous years, the meeting proved to be highly popular and attracted over 140 registrants. The overall intention of the meeting was to alert researchers to emerging areas of chemistry and novel classes of compounds for a range of enzyme and receptor targets likely to lead to new approaches to disease treatment.

The symposium opened with a description by Dr Hazel Hunt (Celltech Chiroscience, Cambridge) of a novel class of phosphodiesterase (PDE) inhibitors. PDE enzymes are responsible for the hydrolysis of ubiquitous second messengers cAMP and cGMP to inactive AMP and GMP, respectively. Inhibition of PDE enzymes results in an accumulation of cyclic nucleotides, which leads to a modulation of the activation state of a number of biological pathways. The selective inhibition of a specific PDE isoenzyme has been an objective of the pharmaceutical industry for many years. Selective PDE3, PDE4 and PDE5 inhibitors are well documented and are exemplified by drugs already launched as well as many compounds undergoing clinical evaluation. PDE enzymes can be classified into 11 isoenzyme families based on substrate specificity and sensitivity to endogenous and exogenous regulators, but physiological roles have not been defined for all of them (e.g. PDE8-11). PDE7 is a cAMP-specific enzyme which has been shown to be insensitive to most standard PDE inhibitors, such as the PDE4 inhibitor, rolipram. Human PDE7 mRNA exists as two splice variants: PDE7A1 is widely distributed and PDE7A2 exists in skeletal muscle, heart and kidney, but active PDE7A protein has been detected primarily in T-cells. Recently, the presence of PDE7 in airway epithelial cells has also been reported. This distribution suggests that selective PDE7 inhibitors may have utility in T cell mediated diseases and disorders of the airways. Hut78 cells have been shown to produce PDE7 and can be used in screening. Two series of selective inhibitors were described. The guanine series (1) have low μM IC_{50}s and show little activity against isoenzymes 3 and 4. The SAR investigation showed that an 8-bromoguanine was preferred and a range of substituents were tolerated in the aromatic ring of the tetrahydronaphthalene, with 8-Br (1μM) and 7-CO_2Me (1.4μM) being the optimum. A second series of sulphonamides (2) produced sub-micromolar selective compounds but, for intellectual property reasons, the structures of the most potent compounds could not be revealed. The SAR investigations showed that replacement of the sulphonamide linkage with amides, amines or ureas reduced inhibitory potency. The 3-OH, 4-OMe combination was one of the best and was used to investigate substitution in the indoline ring. The indoline could be replaced with a tetrahydroquinoline. Substituents at the 5 position of the indoline were preferred with -NHCOPh (2) giving a compound with an IC_{50} = 0.36μM and a selectivity of tenfold over other isoenzymes. Three undisclosed compounds (63597, 63627, 63705) were slightly more potent and more selective. No comment was made on the biological effect of the compounds on T-cells.

The symposium included two talks on the design of selective ligands for the adenosine receptors. Adenosine is involved in an enormous range of physiological processes and mediates its biological activity via the family of P1 purinergic receptors. This family consists of four G-protein coupled 7-trans-membrane receptor sub-types (A_1, A_{2a}, A_{2b} and A_3) and the identification of selective ligands for these subtypes has

been the objective of various research groups. Much effort has been devoted to define the pharmacology and tissue distribution of the various receptors, and thus deduce any possible disease indications. The design of selective ligands to accelerate the biological understanding of this receptor family is an important part of the of the target validation process. Dr Rick Cousins (GlaxoWellcome, Stevenage) gave the first of the talks. He described studies towards the preparation of novel purinyl-based structures and the strategies used in their synthesis. Particular attention was given to illustrate how modifications of the ribose moiety and substituents on adenosine generated potent selective purinergic receptor agonists. Array methodology was used to introduce a variety of lipophilic amine substituents into the adenosine ring at positions 2 and 6. The D-ribose ring was retained throughout. A series of potent A_{2a} agonists (typified by 3) was identified, with subnanomolar binding affinities *in vitro* (measured against human receptors in a CHO cell line) and selectivities over A_1 and A_3 greater than 100. No comment was made for affinity or selectivity at A2b receptors.

The second talk on adenosine receptor ligands was given by Dr Lars Knutsen (Vernalis, Winnersh). The majority of the work described was carried out at Novo Nordisk. The novel adenosine A_1 agonists NNC 21-0136 and NNC 21-0147 constitute new biological leads as anti-ischaemic agents with neuro-protective effect in both global and focal rodent ischaemia models. These compounds are potential drug candidates for stroke since they possess diminished cardiovascular effects in rats when compared to reference A_1 agonists such as *N*-cyclopentyladenosine and *R*-phenylisopropyladenosine. The presentation revealed the screening plan and brief SAR for these new series of P1 agonists. In the NNC 21-0136 series one SAR was observed for the affinity of these nucleosides to the rat brain A_1 receptor, but another SAR for the diminished cardiovascular effects. The SAR of 9-cyclo-pentylpurines was investigated. The purine 6- and 9-position SAR revealed that compounds such as NNC 53-0016 (456nM at A3) were an extension of an earlier *N*-alkoxyadenosine series of A_3 agonists. These latest compounds also possess cytokine modulating properties (down regulation of TNF-alpha gene expression) and some examples were found to be novel, potent inhibitors of phosphodiesterase type IV.

Dr Laurent Hennequin (AstraZeneca, Reimes, France) described the ongoing work that has led to the design of selective inhibitors of VEGF (Vascular Endothelial Growth Factor) receptor tyrosine kinase, as potential angiogenesis inhibitors. These will be important because pathological angiogenesis has been associated with a variety of disease states including diabetic retinopathy, psoriasis, cancer and rheumatoid arthritis. VEGF is a key mediator of angiogenesis implicated in tumour blood vessel formation, a prerequisite for the growth and metastasis of solid tumours. Inhibition of VEGF receptor-associated tyrosine kinase (RTK) activity should abolish VEGF signalling. At the molecular level, VEGF is a ligand for two VEGF RTKs: Flt-1 and KDR. Screening against these target enzymes — and in a whole cell assay — VEGF-driven proliferation of human umbilical vein vascular endothelial cells (HUVECs), as well as the selectivity profile in a panel of kinases led to the identification of ZD4190, an aminoquinazoline inhibitor of KDR activity amenable to oral administration. Homology models of the ATP binding site showed a key N1 H-bond interaction. *In-vivo* activity in disease models (human xenografts) was seen with tumour growth from a variety of tissues: colon, lung, breast, prostate and ovary, at 100mg/kg/d. Pharmacokinetic studies showed that while the compound had an acceptable $t_{1/2}$ (~1.5h) and good bioavailability (F% >80) in the rat, bioavailability was much lower (F% <20) in dog and monkey. Analysis of the physicochemical properties, however, showed that the solubility at pH 7.4 was only 0.7μM and thus, ZD4190 was dropped.

Improved solubility was found by introducing an alternative alkoxyamino substituent to give ZD6474, which had solubility >500μM at pH 7.4. Good bioavailability was observed in rat and dog. There was no sacrifice of activity against tumour growth. The compound is in Phase I clinical trials.

Targeting RNA as a drug target is a concept that has been around for some time, and the antisense approach to RNA has been the subject of considerable effort over a number of years. Dr Justin Bower (RiboTargets, Cambridge) gave an interesting overview of the current position and demonstrated that small-molecule inhibitors of RNA-protein interactions are now realistic targets. The position has changed because the intricacies of RNA tertiary structure have recently been revealed. Using high-resolution NMR and X-ray crystallographic data, RiboTargets is focusing on the development of small molecule inhibitors of RNA for anti-infective targets. Dr Bower described work in two areas.

First, he showed some progress in the HIV area. TAR is a structured RNA element found at the 5' end of all HIV-1 viral transcripts. Upon binding the transcription factor protein Tat, the processivity of transcription is enhanced, leading to full-length viral transcripts and ultimately viral protein. Blocking this Tat/TAR interaction has been shown to prevent viral protein synthesis. Using high-resolution NMR ternary complexes, the development of a novel series of Tat/TAR inhibitors (4), based on a known lead ALX0019, was achieved. Compounds were assayed in a fluorescence-based assay with RNA as the acceptor and the free peptide as the donor. The initial compounds possessed a substituted guanidine which was successfully replaced by an amino-benzimidazole (5) using 'SAR by NMR', with no loss of potency.

The second example for demonstrating small-molecule RNA-protein inhibitors was in the antibacterial area. The bacterial ribosome has long been a target of interest for novel anti-bacterial agents. Many natural-product antibiotics of significant clinical importance have been shown to interact at the ribosomal RNA (rRNA) level, including macrolides, aminoglycosides, streptogramins and tetracyclines. Most recently the non-natural oxazolidinone antibiotics have also been shown to act at the level of rRNA. Until recently, rational structure based design on rRNA has been hampered by the availability of high-resolution structural data; however, the recent publication of high-resolution X-ray structures of fragments of rRNA, and indeed of the 50S and 30S subunits, are set to revolutionise this area. RiboTargets developed an *in-silico* virtual screening technology (RiboDock™) which is capable of high-throughput docking against RNA/protein targets. Efforts targeting the thiostrepton binding sight on the 50S subunit were described. 1.2 million vitrual structures were docked of which 0.1% were virtual hits. One hundred and ninety-seven compounds could be purchased, of which 13 had acceptable activity in GTPase-activating region (GAR) binding assay at 50μM. Of these, three had acceptable activity and specificity in a bacterial translation assay. One of these, RBT1161 (no structure given) had comparable activity (20.5μM) with an oxazolidinone (6) (11μM). Both are prokaryote specific. RBT1161 has MIC of 11.8μg/ml against MRSA. The whole process for identification of the lead took six to eight weeks.

Continuing the anti-infectives theme, Dr Andy Bell (Pfizer, Sandwich) gave a presentation on the design of inhibitors for protein N-Myristoyl transferase (NMT), an enzyme found in all eukaryotic cells, which catalyses the transfer of the rare C_{14} fatty acid, myristic acid, to the N-terminal glycine of its substrate proteins. The need is for a fungicidal agent rather than a fungistatic. In collaboration with Millennium, gene knock-out experiments in yeast and *Candida* have shown NMT to be an essential enzyme, and that temperature-sensitive dependent shut-off leads to cidality; therefore, NMT is an attractive antifungal target. Despite extensive elegant efforts, particularly by the Searle group, only weakly

antifungal NMT inhibitors have been identified to date. It was hypothesised that the weakness of antifungal activity of previous NMT inhibitors could be due to their large peptidomimetic nature and, consequently, Pfizer decided to look for alternative starting-points through a high throughput screen of their compound file. CP-123,457 was identified as an inhibitor of *Candida albicans* NMT (IC_{50} = 1.4μM) and it proved amenable to rapid follow-up by parallel synthesis. They were able to replace the potentially vulnerable ester functionality and achieve increased enzyme potency. Lipophilicity was increased by derivatising the primary amine and although enzyme potency was reduced with small alkyl groups, reductive amination with aryl aldehydes gave several analogues with increased enzyme potency, for example, UK-362,091 (IC_{50}= 11nM, Log D=3.4) and antifungal activity, with a fungicidal mechanism of action. Several other primary amines, for example UK-370,485 (IC_{50}=42nM) were made. A crystal structure of the binary complex of *Candida albicans* NMT with UK-370,485 was determined (2Å) to reveal a tight network of hydrogen bonds around the primary amino group that suggested a possible mechanism for the myristoylation of substrate proteins. Additional isothermal calorimetry experiments were used to investigate the thermodynamics of the binding to the enzyme. The experiments suggested that a ternary complex was involved involving myristoyl-CoA, and that UK-370,485 binds with a strong enthalpic contribution with loss of entropy on binding. Despite their excellent enzyme potency, the primary amine series was felt to be too polar for good cellular penetration (Log D=-0.8 to 0). Alkylation of the amine, as in UK-362,091 gave UK-370,753 (IC_{50} = 86nM, MIC 0.09μg/ml) which is more active antifungally than versus the enzyme. It has a fungicidal mode of action.

In contrast to presentations on synthetic compounds, Dr Colin Edge (SmithKline Beecham, Harlow) described computational chemistry approaches that have been taken to enhance screening collections. He showed that compounds designed and marketed for human disorders differ in their physical properties from antibacterial drugs. Analysis of physical properties of marketed drug sets allows the devising of simple rules, similar to the 'Rule of 5' proposed by Lipinski et al., which delimit the boundaries of property space for certain therapeutic classes. A set of rules were devised for antibacterials: molecular weights should be in the range 300–600; MlogP <3 (Moriguchi LogP was chosen over cLogP because it is more robust despite being less accurate, it has fewer fiddle factors and is a simple summation of atomic contributions), H-bond donors <10 and acceptors <15. The drug sets were also broken down into constituent functional groups in an attempt to make a drug-like scoring system. The most common fragment of CNS drugs is a tertiary amine, whereas for antibacterials, polar groups appear most. The rules and scoring system have been used to buy compounds to enhance the SB collection and to design combinatorial libraries which are focused towards particular therapeutic classes.

Dr Francis Wilson (Roche, Welwyn Garden City) talked on the design and synthesis of inhibitors to combat hepatitis-C. Hepatitis-C Virus (HCV) is the cause of the majority of cases of transfusion-associated hepatitis and a significant proportion of community-acquired hepatitis worldwide. Infection by HCV can lead to a range of clinical conditions including an asymptomatic carrier state, severe chronic active hepatitis, cirrhosis and, in some cases, hepato-cellular carcinoma. Current estimates show ~300 million chronically infected worldwide.

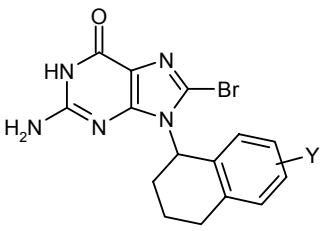
The virus possesses a chymotrypsin-like serine proteinase, NS3, which has protease and helicase function. The protease typically cleaves after a cysteine. The first-generation electrophile-based inhibitors designed by Roche were aldehyde and boronic acids which had hepatotoxicity issues. Rat hepatocyte cultures were used to profile compounds for potential hepatotoxicity. Replacement of a boronic acid with the alpha-keto

amide (for example, Ro 36-3810/000) gave a hepatocyte penetrant compound (IC_{50} 4nM) that causes less of a rise in **lever** enzymes than the boronic acids. The key issue, however, is insufficient selectivity over other serine proteases (for example, chymotrypsin, elastase).

Work on antidepressants

There is a clear need for an antidepressant compound with a faster onset of therapeutic action. Dr Monique van Niel (Merck Sharpe & Dohme, Harlow) described research designed to generate such compounds. They are designed to have dual activity against the 5-HT_{1A} receptor and the 5-HT neurotransmitter (re-uptake) transporter (5-HTT) and should increase the amount of 5-HT released in synapses of the brain. The hypothesis is based on clinical observations that suggested a greater proportion of patients with depression responded when the 5-HT_{1A} partial agonist pindolol was co-administered with a selective serotonin re-uptake inhibitor (SSRI), compared with those receiving only SSRIs, and that a reduced treatment time was needed to obtain a sustained response. The assumption is that elevated levels of 5-HT are a contributing factor. Merck generated relevant biological data using the following systems: cloned 5-HT_{1A} receptors (Hela cells) (binding), 5-HTT (HEK cells) (binding), agonist-induced GTPγS binding (function), inhibition of PCA-induced 5-HT depletion in rat (function), displacement of tritiated MPPF to determine 5-HT_{1A} receptor occupancy in mouse cortex (function), and *in-vivo* dialysis in rat hippocampus to measure 5-HT efflux. SAR on a series of amino alcohols, based on a compound from Eli Lilly, were described, which led to (7). The S enatiomer at the secondary alcohol centre was preferred. The compound had reasonably high clearance, good bioavailability and a brain: blood distribution ratio of 3: 5. Unfortunately, these compounds had some alpha1A affinity. A second series, based on a lead from Bristol Myers Squibb, led to L792, 239. L792, 239 has high affinity at 5-HT_{1A} receptors (1.3nM) and the re-uptake site (0.1nM) and an intrinsic activity of 35%. *In-vivo* microdialysis experiments on this compound, which measured the amount of 5-HT efflux, indicated that levels were equivalent to those obtained using a combination of fluoxetine and the 5-HT_{1A} antagonist WAY 100, 635; thus, in line with expectations. Alternative series of compounds are also being investigated.

In summary, this was a popular, well-organised meeting with the nine speakers representing nine different pharmaceutical organisations. In addition to the continuing work on well-established classes of targets, new and exciting opportunities were presented based on new information from genomic and structural sources, in addition to approaches based on good clinical observation.



Y = 8-Br or 7-CO_2Me

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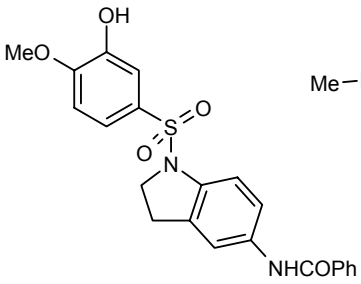

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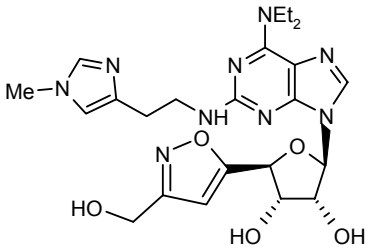

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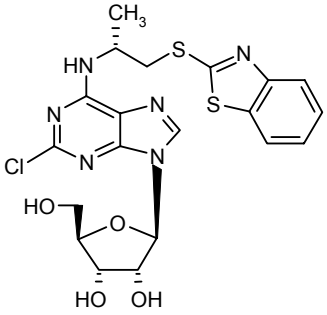

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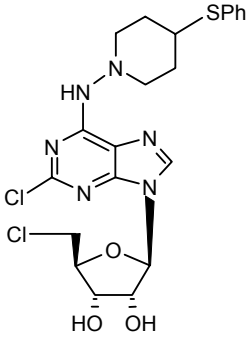

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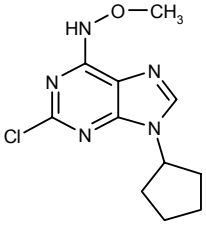

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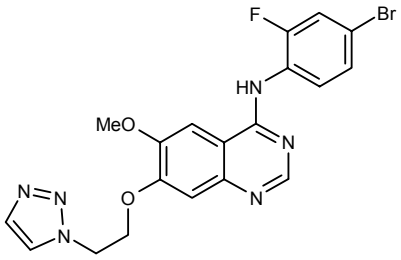

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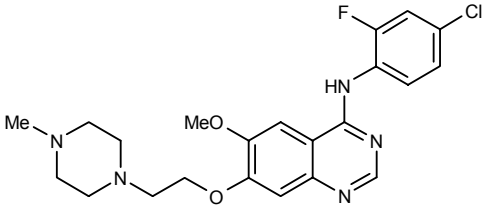

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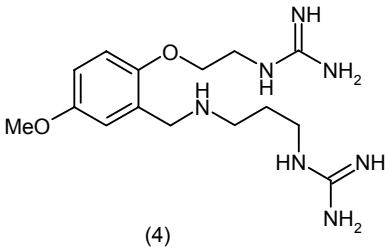

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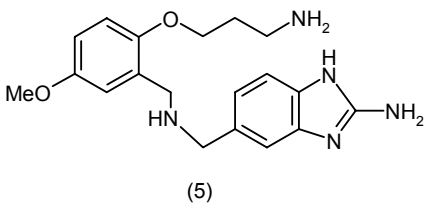

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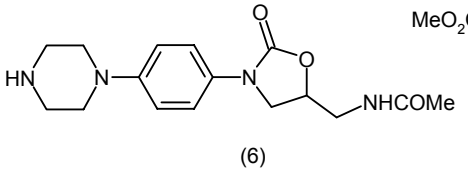

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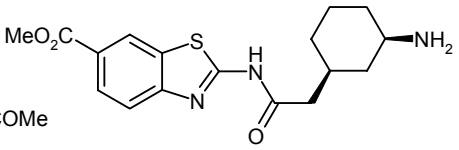

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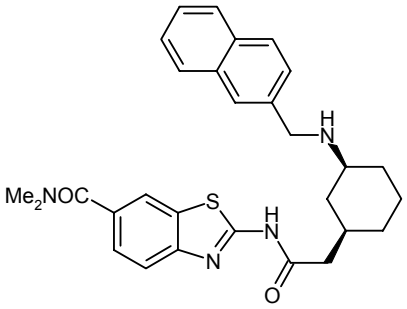

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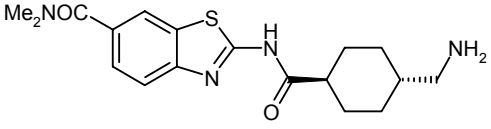

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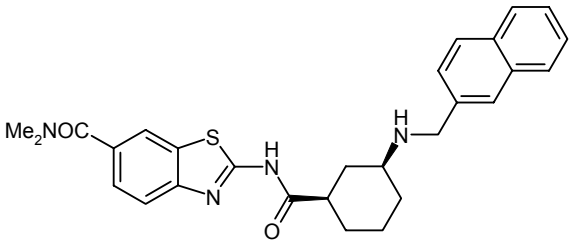

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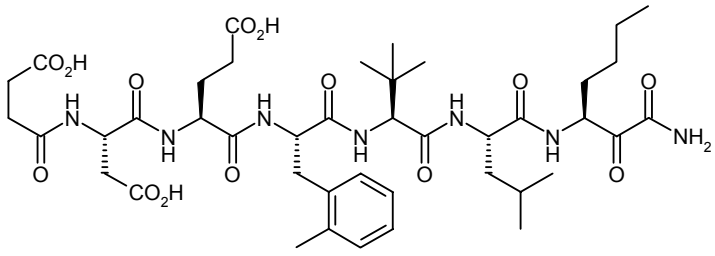
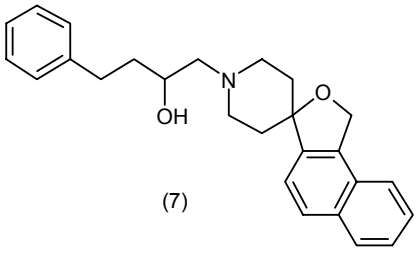


Ro 36-3810/000



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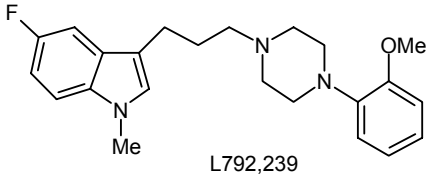

L792,239