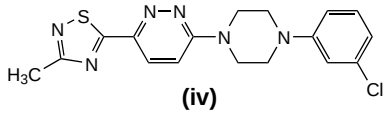
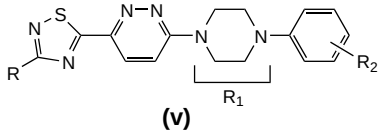
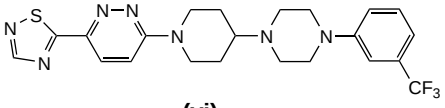


(iv)



(v)



(vi)

controls. Ocular toxicity was assessed after oral dosing of the compounds at 40 mg kg^{-1}, twice a day for four days, in male Wistar rats. Finally, *in vivo* antitumor and antimetastatic activities were measured after oral dosing, twice a day for 28 days, to B6D2F1 mice bearing metastatic 3LL Lewis lung tumors. The results clearly showed that: (1) the pyridazine ring is nearly optimal for primary anti-angiogenic activity; (2) replacement of the pyridazine ring by other aromatic cyclic structures was sufficient to eliminate eye toxicity; (3) replacement of the methyl substituent at the 3-position of the thiadiazole ring by other groups, while keeping the pyridazine moiety, eliminated eye toxicity (although loss of activity was also seen); and (4) the substituted phenyl ring is essential for activity. The optimal phenyl substituents are electron-withdrawing groups, preferably at the *meta* position.

Based on these results, promising compounds were obtained by avoiding the 3-methyl substitution of the thiadiazole ring and by modifying the central piperazine spacer. Among others, compound **(vi)**, which has no eye toxicity, showed an IC_{50} value of 0.3 nM and 54% inhibition. Attempts to characterize the mode-of-action of this series have failed until now, although they did prove to be inactive against a panel of tyrosine and serine or threonine kinases including VEGF and EGF. They are also inactive against the matrix metalloproteinases 1–3 and 7–9, and other proteolytic enzymes, such as urokinase, calpain, tissue kallikrein and chymotrypsin.

4 Auerbach, W. *et al.* (1994) Angiogenesis inhibition: a review. *Pharmacol. Ther.* 63, 265–311

5 Deplanque, G. *et al.* (2000) Anti-angiogenic agents: clinical trial design and therapies in development. *Euro. J. Cancer* 36, 1713–1724

6 Nicosia, R.F. *et al.* (1990) Growth of microvessels in serum-free matrix culture of rat aorta. A quantitative assay of angiogenesis *in vitro*. *Lab. Invest.* 63, 115–122

7 Bongartz, J-P. *et al.* (2002) Synthesis and anti-angiogenic activity of 6-(1,2,4-thiadiazol-5-yl)-3-amino pyridazine derivatives. *Bioorg. Med. Chem.* 12, 589–591

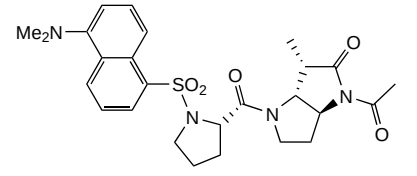
Daniela Barlocco
University of Milan
Viale Abruzzi 42, Milano-20131, Italy
tel: +39 02 2950 2223
fax: +39 02 2951 4197
e-mail: daniela.barlocco@unimi.it

Novel antiviral molecules

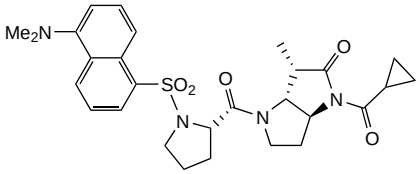
HCMV-protease inhibitors

Human cytomegalovirus (HCMV) expresses a unique serine protease, which is essential for the lifecycle of the virus. What makes this protease unique is that, instead of the usual Asp-His-Ser catalytic triad, which is found in most trypsin-like proteases, the HCMV serine protease active site is composed of a His-His-Ser triad. Thus, the HCMV serine protease has become an attractive target for the design of antiviral agents.

Borthwick *et al.* from the laboratories of GlaxoSmithKline (Stevenage, UK) report the discovery and SAR of a novel series of mechanism based inhibitors of the HCMV-protease [1]. The most potent compounds arrived at are **(i)** and **(ii)** with IC_{50} values of 0.54 and 0.34 μM, respectively. In this study, the steric and stereochemical requirements for the substituent α to the lactam carbonyl were determined. In addition, the substituents projecting from the lactam and pyrrolidine nitrogen atoms were optimized. Mechanism studies showed that these inhibitors act by acylating the active-site serine residue (Ser 132) in a time-dependent and reversible manner.



(i)



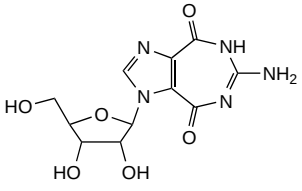
(ii)

1 Borthwick, A.D. *et al.* (2002) Design and synthesis of pyrrolidine-5,5-*trans*-lactams (5-oxohexahydropyrrolo[3,2-*b*]pyrroles) as novel mechanism-based inhibitors of human cytomegalovirus protease. 2. Potency and chirality. *J. Med. Chem.* 45, 1–18

Purine-ring modified inhibitors of HBV

An estimated 350 million people worldwide are chronically infected with the hepatitis B virus (HBV), a number of which die each year because of complications. In addition, chronic infection with HBV is associated with hepatocellular carcinoma. Unfortunately, at present there exists only one therapy approved for the treatment of HBV, 2′,3′-dideoxy-L-3′-thiacytidine (3TC).

A recent report describes a class of nucleoside analogues, which are modified in a unique manner and are active against HBV replication in cell culture [2]. The compounds are ring-expanded nucleoside analogues, wherein the heterocyclic base has been modified as opposed to the sugar, which is the usual target of modification. Compound **(iii)** is a representative example and is the most active compound disclosed. It was found to inhibit the formation of HBV-virions in infected 2.2.15-cells (HBV virion producing cells) with an EC_{50} value of 0.13 μM.



(iii)

1359-6446/02/$ – see front matter PII: S1359-6446(02)02285-7

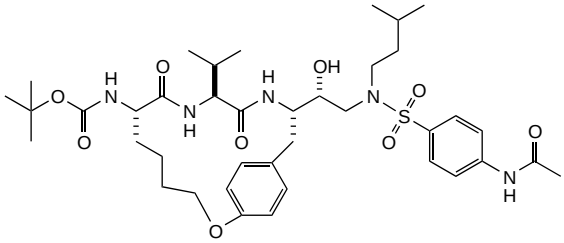
An interesting feature of this chemotype is that the ribose sugar is required for activity: the 2′-deoxy analogue has no activity against HBV. Finally, compound **(iii)** was found to be selective for HBV over other viruses, such as HIV, HSV, CMV (cytomegalovirus), VZV (varicella zoster virus) and EBV (Epstein-Barr virus), and was minimally toxic to non-infected cells.

2 Sood, R.K. *et al.* (2002) Novel ring-expanded nucleoside analogs exhibit potent and selective inhibition of hepatitis B virus replication in cultured human hepatoblastoma cells *Antiviral Res.* 53, 159–164

Cyclic-peptide based HIV-protease inhibitors

Proteases usually bind their substrate peptides in β-sheet structures. HIV-protease is no exception and this principle can be used in the design of peptomimetic inhibitors of the enzyme. As such, these inhibitors can potentially serve as antiviral therapeutics against AIDS.

Cyclic peptide based inhibitors of HIV protease are the focus of a report from the Center for Drug Design and Development at the University of Queensland, Australia [3]. An example is compound **(iv)** with a K_i value of 2.0 nM against the enzyme and a IC_{50} value of 177 nM against HIV in cell culture. The macrocycle of this peptidomimetic holds the inhibitor in the β-sheet form, mimicking the substrate and thus reducing the entropy loss associated with binding to the active site. Because the entropic energy price is paid before binding it is assumed that there is more tolerance for side-chain fit. The cyclic peptides also have the advantage of being more resistant to *in vivo* degradation and more easily absorbed into the cell than their linear peptide counter parts.



(iv)

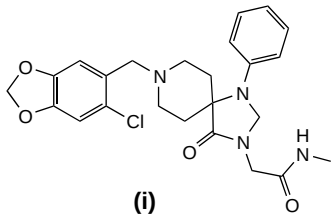
3 Glenn, M.P. *et al.* (2002) β-Strand mimicking macrocyclic amino acids: Templates for protease inhibitors with antiviral activity *J. Med. Chem.* 45, 371–381

Michael A. Walker
Bristol-Myers Squibb
Pharmaceutical Research Institute
Wallingford, CT 06492, USA
tel: +1 203 677 6686
fax: +1 203 677 7702
e-mail: walkerma@bms.com

Combinatorial chemistry

μ Opiate ligands

Parallel synthesis and HTS, which rely to varying degrees on molecular modelling for library management, description, similarity evaluation and quantitative SARs, have led to a dramatic increase in throughput in the primary stages of drug discovery. A generic hit-to-lead strategy, relying on the systematic generation and evaluation of analogues around the parent hit compound, has been reported [1]. The strategy makes use of two different analoging methods to cover efficiently the structural space around hits. The first method is classical exploratory analoging based on similar topology. The second method relies on algorithms for rapid generation of 3D molecular fingerprints based on multi-conformational models of candidate compounds, in combination with similarity evaluation tools for the comparison of 3D fingerprints. To validate this strategy experimentally, the authors have applied this to the search for potent μ opiate ligands. A library of 294 single compounds was synthesized in solution. The library compounds were screened at 10 μM for binding at the μ opiate human receptor. One of the most potent compounds found was **(i)**, (IC_{50} = 0.9 nM against the human μ-opiate ligand). This work has shown that the two design methods indicated could be used in parallel for the elucidation of SARs, and the potent compounds discovered here could be important in further work in this area.



(i)

1 Poulain, R. *et. al.* (2001) From hit to lead. Combining two complementary methods for focused library design. Application to μ opiate ligands. *J. Med. Chem.* 44, 3378–3390

Influenza virus fusion inhibitors

Problematic annual outbreaks of influenza continue to emerge and are associated with significant morbidity and, in certain populations, mortality. These annual epidemics are driven by antigenic drift, a consequence of the poor fidelity of the influenza virus RNA polymerase. The more insidious pandemics, which occur less frequently but impose a much greater disease burden, result from antigenic shift, the production of reassortant viruses. Recently, a strategy of prophylaxis that depends upon vaccination campaigns conducted immediately before the onset of the influenza season has been implemented. However, the success of this approach is dependent upon an ability to accurately predict the anticipated circulating virus several months in advance, to produce an appropriately effective vaccine. As part of an overall effort towards further defining the fusion-inhibiting pharmacophore and identifying compounds with both increased potency and inhibition of H3 influenza subtypes, exploration of SARs associated with the amine element of literature compound **(ii)** (BMY27709 [2], identified as an effective and potent inhibitor of the H1 and H2 subtypes of influenza A virus strain [3]) were performed. Two libraries, giving a total of 418 single compounds, were synthesized

  PII: S1359-6446(02)02269-9