Monitor: molecules and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

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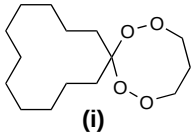
Molecules

Antimalarial activity of acyclic peroxides

Malaria parasites are rapidly developing multidrug resistance to the most common chemotherapeutic alkaloidal drugs. Therefore, interest in the antimalarial properties of nonalkaloidal compounds, such as artemisinin and the related endoperoxides, is rapidly growing [1]. Recently, the 3-cyclododecyl-1,2,4,5-tetroxocane **(i)** and related compounds have been reported [2] to show remarkable antimalaria activity *in vitro* (EC_{50} = 0.025 μM). By contrast, poor attention has been devoted to the acyclic peroxides. Because of this, Nojima and co-workers [3] synthesized a series of 20 acyclic analogues of the endoperoxide **(i)**, either symmetrically or unsymmetrically substituted [compound **(ii)**] together with several α-methoxysubstituted peroxides [compound **(iii)**]. Compounds were tested *in vitro* for their antimalarial activity against *Plasmodium falciparum* and for cytotoxicity against FM3A cells. In addition, the most interesting derivatives were tested *in vivo* against *P. berghei* NK65 strain. Compound **(iia)** (R = R_1= Me) showed an IC_{50} value of 0.086 μM but poor selectivity (17). By contrast, compound **(iiia)** (R = Me), which had similar activity (IC_{50} = 0.080 μM), displayed a better selectivity (238). In the same assay, artemisinin values were IC_{50} = 0.01 μM and the selectivity



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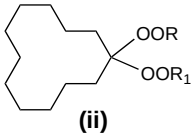

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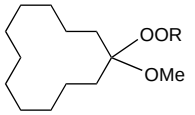

(iii)

was 1000. Altogether, the *in vitro* data proved that both the nature of the substituents on the peroxide and the presence of the cyclododecane ring are important factors for the activity of these series. When tested intraperitoneally against *P. berghei* NK65 strain, compound **(iia)** and artemisinin showed ED_{50} values of 13 mg kg^{-1} and 5.4 mg kg^{-1}, respectively. On the contrary, **(iiia)** showed only moderate activity (ED_{50} = 30 mg kg^{-1}). Finally, it should be noted that compound **(iia)** was found to be orally active (ED_{50} = 30 mg kg^{-1}).

1 Li, Y. *et al.* (2000) Synthesis and antimalarial activity of artemisinin derivatives containing an amino group. *J. Med. Chem.* 43, 1635–1640
2 Kim, H-S. *et al.* (2001) Synthesis and antimalarial activity of 1,2,4,5-tetraoxacycloalkanes. *J. Med. Chem.* 44, 2357–2361
3 Hamada, Y. *et al.* (2002) Synthesis and notable antimalarial activity of acyclic peroxides, 1-(alkyldioxy)-1-(methyldioxy)cyclododecanes. *J. Med. Chem.* 45, 1374–1378

Thiadiazole pyridazine derivatives with potential anti-angiogenic activity

Angiogenesis – the formation of new blood vessels – is a tightly controlled process in healthy tissues. By contrast, uncontrolled hyperproliferation of blood vessels can be observed in diseases such as cancer, diabetic retinopathy and arthritis [4]. Therefore, the hypothesis that tumor growth could be arrested by anti-angiogenic treatment has received much attention, and several anti-angiogenic drugs are currently in clinical trials [5]. Recently, from the screening of the Janssen strategic collection using the rat aortic ring assay, compound **(iv)** (R90324) was shown to have nanomolar potency (IC_{50} = 0.6 nM) [6]. However, *in vivo* profiling of **(iv)** showed that it caused severe eye toxicity in rodent models, which prevented its further use in *in vivo* antitumor xenograft models. Therefore, a synthetic program was initiated to optimize the antiangiogenic properties of **(iv)** and to remove its unwanted side effects. Preliminary results [7] indicated that replacement of the thiadiazolyl moiety by isosteric moieties failed to maintain inhibitory activity. Therefore, systematic modifications of other structural features (R, R_1 and R_2) were considered **(v)**.

All the compounds were tested for their antiangiogenic properties by quantitation of microvessel growth in rat aortic rings cultured in a fibrin clot for eight days, and compared to vehicle-treated

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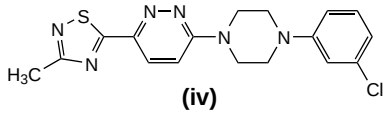

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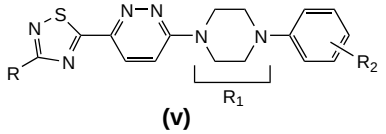

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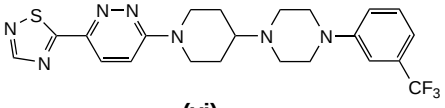

(vi)

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

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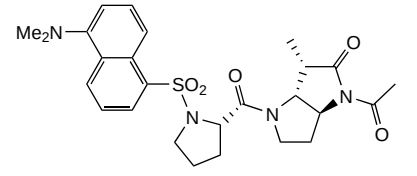
Novel antiviral molecules

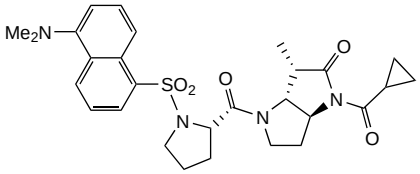
HCMV-protease inhibitors

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)



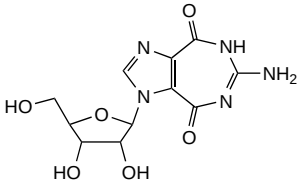
(ii)

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.



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