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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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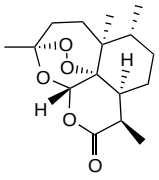
Molecules

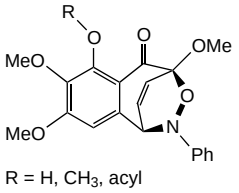
Novel potential antimalarial compounds

Human malaria remains a threat to more than two billion people, living in areas of high incidence. It is estimated that there are 150 million new cases every year. *Plasmodium falciparum,* the causative agent of the most malignant forms of malaria, is a particularly resistant parasite, which is known to have high adaptability by mutation. This mutability increases the likelihood of the development of resistance to vaccines and chemotherapies, including drugs with novel modes of action, such as artemisinin **(i)** (Ref. 1).



(i)

It has been proposed that artemisinin acts by interaction of its reactive endoperoxide bond with active Fe^{2+} species, present in the protozoan vacuole, which catalyzes the generation of highly cytotoxic oxygen- and carbon-centred radicals[2]. The standard bond-dissociation energy (SBD) of the peroxo linkage (-O–O-) and the -N–O- linkage was reported to be 33.2 and 35.8 kcal mol^{-1}, respectively[3]. As a result, Moyna and coworkers reasoned that oxazines could follow a reaction pattern (i.e. -N–O- bond opening and radical generation) similar to that of endoperoxides. However, the differences in chemical structure could make these compounds unaffected by the potential *Plasmodium* variants capable of inactivating endoperoxides. Therefore, they prepared a series of oxazines based on **(ii)** and tested them for their *in vitro* activity against the protozoan parasites *P. falciparum* and *Trypanosoma cruzi*. In addition, their toxicity was evaluated against KB (epithelial) cells[4]. Although all the compounds were at least three orders of magnitude less active than chloroquine (IC_{50} = 3.0 nM) in the *P. falciparum* assay, most of them displayed activities in the micromolar concentration range. However, several compounds exhibited activity comparable to benznidazole (ED_{50} = 7.1 μM) against *T. cruzi.* It should be noted that, although many derivatives were highly toxic in the KB cell assay, several compounds with the highest activities had low or moderate toxicity. This seems to indicate that activity is independent from toxicity. The most interesting compound was the derivative of **(ii)** in which R = CH_3COOCH_2CO, which had the lowest toxicity (ED_{50} = 128.2 μM), one of the poorest activities against *T. cruzi* (IC_{50} > 63.9 μM), and the highest activity against *P. falciparum* (IC_{50} = 3.2 μM). It has been speculated that this compound might behave as a prodrug.



R = H, CH_3, acyl

(ii)

1 Enserink, M. (2000) Malaria researchers wait for industry to join fight. *Science* 287, 1956–1958

2 Meshnick, S.R. *et al.* (1996) Second-generation antimalarial endoperoxides. *Parasitol. Today* 12, 79–82

3 Pauling, L. (1960) *The Nature of the Chemical Bond* (3rd edn), Chapter 3, Cornell University, Ithaca, NY, USA

4 Ren, H. *et al.* (2001) Design, synthesis, and biological evaluation of a series of simple and novel potential antimalarial compounds. *Bioorg. Med. Chem.* 11, 1851–1854

Novel potent and selective nAChR ligands derived from cytisine

There is accumulating evidence that ligands acting as an agonist of nicotinic acetylcholine receptors (nAChRs) could be used in the treatment of various neurological disorders related to a decrease in cholinergic function. Although several promising ligands for nAChRs have been developed, there is still a need for potent and selective agents, devoid of the side effects that accompany the naturally occurring prototypical agonists for

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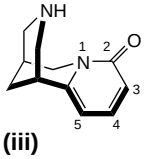
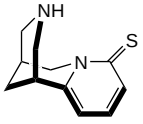
nAChRs, namely (–)-nicotine or (–)-epibatidine. (–)-Cytisine, **(iii)**, which can be easily obtained by extracting from the seeds of *Laburnum anagyroides medicus*, shares various physiological properties with (–)-nicotine[5]. However, although it is acutely toxic in comparison to (–)-nicotine, it is a more potent nAChR ligand and displays higher selectivity toward the α4β2 nAChR subtype and sub-nanomolar affinity. In addition, cytisine has a longer half-life *in vivo* than nicotine[6].



(iii)

Based on these findings, Pujol and coworkers have recently started a programme that aims to identify novel nAChR agonists, structurally related to **(iii)**, which might provide an improved pharmacodynamic profile and safety over the natural alkaloid[7]. In particular, the influence of halogen substituents (e.g. chlorine, bromine and iodine) on the *in vitro* affinity for α4β2 and α7* nAChRs was examined. In addition, the bioisosteric replacement of the lactam by a thiolactame functionality was considered. All the compounds were tested for their *in vitro* affinity for α4β2 and α7* nAChRs by radioligand-binding assays, using (±)-[^{3}H]-epibatidine and [^{3}H]-MLA, respectively. The data showed that the introduction of halogen substituents generally resulted in affinity retention. In particular, the 3-halocytisines showed subnanomolar affinity (K_i values of 0.01–0.022 nM) to central α4β2 nAChRs (Br > I > Cl > H). The most active derivative was the bromoderivative, which had an affinity approximately one-order higher than cytisine (K_i = 0.122 nM) and comparable to that of (±)-epibatidine (K_i = 0.008 nM). Substitution at the 5-position as well as disubstitution at the 3,5-positions gave less favourable results. For the 3,5-substituted derivatives, the K_i values ranged from 0.520 nM (I) to 10.8 nM (Br). It should be noted that the 3-halocytisines also exhibited the highest affinity for the α7* subtype in this series, comparable to that of MLA. Also in this case, the other halo-derivatives were less potent.

The bioisosteric replacement of the lactam oxygen by sulfur **(iv)**, caused a sevenfold lower affinity compared with cytisine, retaining subnanomolar-binding affinity for the α4β2 receptor (K_i = 0.832 nM). Remarkably, this compound had the best affinity selectivity profile (K_i = 4 μM for the α7* subtype). Because the C=S···HN hydrogen bonds are reasonably weaker than their C=O···HN analogues, this could account for the lower affinity of **(iv)** for α4β2 and for the dramatic loss of affinity for the α7* nAChR subtype.



(iv)

5 Anderson, D.J. *et al.* (1994) Nicotinic receptor binding of [^{3}H]cytisine, [^{3}H]nicotine and [^{3}H]methylcarbamylcholine in rat brain. *Eur. J. Pharmacol.* 253, 261–267

6 Sloan, J.W. *et al.* (1988) The comparative binding characteristics of nicotinic ligands and their pharmacology. *Pharmacol. Biochem. Behav.* 30, 255–267

7 Imming, P. *et al.* (2001) Syntheses and evaluation of halogenated cytisine derivatives and bioisosteric thiocytisine as potent and selective nAChR ligands. *Eur. J. Med. Chem.* 36, 375–388

New insulinomimetic Zn^{2+} complexes

Zinc is known to be an essential trace element found in biological systems. Among many other pharmacological and nutritional roles, the Zn^{2+} ion has been reported to act as an insulinomimetic. However, its physicochemical properties are entirely different from vanadium ions, which also show insulinomimetic activity[8].

Recently, Kojima and coworkers[9] reported on a series of Zn^{2+} complexes as possible insulinomimetic agents devoid of severe side effects, based on the general assumption that Zn^{2+} ion is less toxic than vanadium ions[10,11]. The same group had previously discovered that Zn^{2+} maltolate complex with a $Zn(O_4)$ coordination mode was a good insulinomimetic *in vitro*[12]. On this basis, the structures of several Zn^{2+} complexes were examined, and it was found that Zn^{2+} complexes with $Zn(N_2O_2)$ coordination mode exhibit higher insulinomimetic activity with respect to $ZnSO_4$ or $VOSO_4$. All of the complexes with natural amino acids and their derivatives were purified by crystallization from hot water, and were found to be molecular complexes without a counter-ion. Their insulinomimetic activity was evaluated on the isolated rat adipocytes, treated with epinephrine, and compared with that of $ZnSO_4$ and $VOSO_4$. The results clearly indicated that the inhibitory effects of the complexes with overall stability constants (logβ) <10.5 had higher or comparable insulinomimetic activity than the references, with only one exception. By contrast, complexes with higher logβ values did not show any activity. It is interesting to note that the geometry around the Zn^{2+} ion in active and inactive complexes is similar. In addition, when the L-amino acid was substituted by the corresponding D-isoform, similar activities were seen. This suggests that the activity of these compounds is dependent on their logβ value.

The most active compound [Zn(L-Thr)$_2(H_2O)_2$, logβ = 8.46] was also evaluated *in vivo* for its activity in lowering blood-glucose levels in the KK-A^y mouse diabetes model. Results showed that, when this compound was given at a daily dose of 3 mg of Zn per kg of body weight, the glucose levels were lowered and maintained at approximately 220–230 mg dl^{-1} (12.2–12.8 mM) for two weeks. No renal or liver disturbances were noticed, compared with the untreated mice.

8 Coulston, L. *et al.* (1980) Insulin-like effect of zinc on adipocytes. *Diabetes* 29, 665–667

9 Yoshikawa, Y. *et al.* (2001) New insulinomimetic zinc(II) complexes of α-amino acids and their derivatives with $Zn(N_2O_2)$ coordination mode. *Chem. Pharm. Bull.* 49, 652–654
10 Smith, K.T. *et al.* (1988) *Trace Mineral in Foods*, p. 209, Marcel Dekker
11 Smith, K.T. *et al.* (1988) *Trace Mineral in Foods*, p. 257, Marcel Dekker
12 Yoshikawa, Y. *et al.* (2000) New insulin-mimetic zinc (II) complexes; *bis*-maltolato zinc (II) and *bis*-2-hydroxypyridine-*N*-oxido zinc (II) with $Zn(O_4)$ coordination mode. *Chem. Lett.* 874–875

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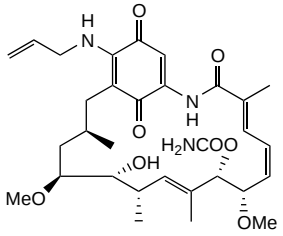
Novel antitumour molecules

A small-molecule Hsp90 inhibitor inducing Her2 degradation

The Hsp90 (heat-shock protein) family of molecular chaperones play a key role in the ATP-dependent refolding of denatured proteins and the conformational maturation of a variety of key cell-signalling proteins that are frequently overexpressed in human cancers, including Raf and some tyrosine kinases (e.g. Met and Her2). In addition, Hsp90 is thought to play a role in maintaining the stability and function of mutated form of proteins such as p53 and *v*-src, but is required to a much lesser extent or not at all for their wildtype counterparts. Hsp90s have also been shown to be overexpressed in multiple tumour types, and cancer cells seem to be especially sensitive to Hsp90 inhibition. For these reasons, Hsp90 would appear to be an attractive target for anticancer therapeutic intervention.

The ansamycin antibiotics geldanamycin, herbimycin and radicicol have been found to cause Hsp90 inhibition by binding in the Hsp90 *N*-terminal ATP/ADP-binding pocket, and a synthetic analogue, 17-allylaminogeldanamycin **(i)** is currently in Phase I clinical trials as an Hsp90 inhibitor. However, **(i)** is relatively insoluble in formulatable solvents, is not easily synthesized and is unselective among the family of (at least four) Hsp90 family members. Chiosis and coworkers at the Memorial Sloan-Kettering Cancer Center (New York, NY, USA) have used the structural features of the Hsp90 binding pocket to design a small-molecule purine-derived Hsp90 inhibitor, PU3 **(ii)** (Ref. 1). This was found to bind competitively with geldanamycin for Hsp90 and induce protein degradation, including Her2, in a similar manner to geldanamycin. PU3 was also found to inhibit the growth of breast cancer cells, causing retinoblastoma protein hypophosphorylation, G1 arrest and differentiation, and thus could provide a new strategy for cancer treatment.



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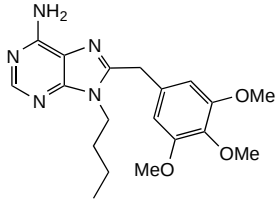
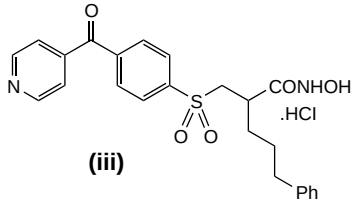


(ii)

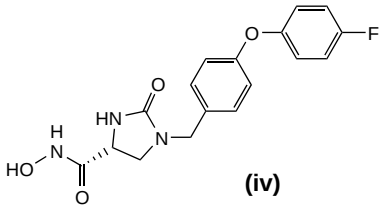
1 Chiosis, G. *et al.* (2001) A small molecule designed to bind to the adenine nucleotide pocket of Hsp90 causes Her2 degradation and the growth arrest and differentiation of breast cancer cells. *Chem. Biol.* 8, 289–299

Selective matrix metalloproteinase inhibitors

There is a well-established association between matrix metalloproteinase (MMP) overexpression and diseases such as metastatic cancer and arthritis. Despite intensive research efforts, the clinical development of MMP inhibitors (e.g. Marimastat) has been hampered by side effects such as musculoskeletal pain and stiffness (limiting the maximum tolerable dose), which are thought to arise at least in part from activity against the shedding of ectodomain proteins, or so-called 'sheddase' activity. Watson and coworkers at Celltech Chiroscience (Cambridge, UK) have used computer-aided molecular design techniques to search databases for non-peptidic selective inhibitors of MMP-8, based on the MMP-8 crystal structure[2]. Several modestly potent inhibitors were identified, from which chemical lead-optimization produced potent MMP-8 inhibitors, such as **(iii)**. Compound **(iii)** was found to inhibit MMP-8 with an IC_{50} value of 3 nM (together with MMP-2 and MMP-9 in the nanomolar range) when tested against a range of MMPs. Encouragingly, no activity against the shedding of ectodomain proteins was observed.



(iii)



(iv)

In related work, Robinson and coworkers at Pfizer Global Research and Development (Groton, CT, USA) have described the design, synthesis and evaluation of a series of imidazolidinone-based compounds as MMP-13 inhibitors[3]. The most notable finding amongst these was derivative **(iv)**, which selectively inhibited MMP-13 with an IC_{50} value of 3 nM.

2 Baxter, A.D. *et al.* (2001) Arylsulphonyl hydroxamic acids: potent and selective matrix metalloproteinase inhibitors. *Bioorg. Med. Chem. Lett.* 11, 1465–1468

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