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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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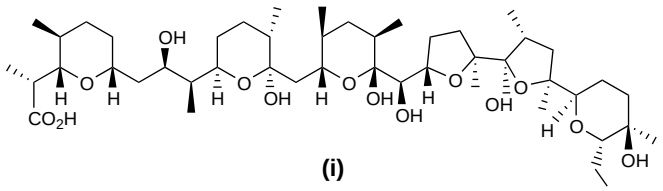
Molecules

A novel potent antimalarial natural product

Malaria is still a major cause of death in tropical regions. In a single year, there are generally >500 million clinical cases of infection resulting in 2.7 million deaths. Additionally, the growing resistance of the infectious agent to currently available antimalarials, such as chloroquine, indicates that novel strategies and chemical entities are urgently required to improve control of this disease. Recently, researchers at the Kitasato Institute (Tokyo, Japan) have identified the polyether antibiotic **(i)** as a potent and selective inhibitor of drug-resistant and drug-sensitive strains of *Plasmodium falciparum* [1].

During a screening program to discover novel antimalarial antibiotics from soil microorganisms with both *in vitro* and *in vivo* activity against drug resistant malaria parasites, the known polyether antibiotic X206 **(i)** was identified from the culture broth of the actinomycete strain K990413. The potent *in vitro* antimalarial activity of compound **(i)** against the K1 strain of *P. falciparum* (drug-resistant) was indicated by an IC_{50} value of 0.15 nM, in comparison to 7.6 nM for artemether, the most potent clinically used antimalarial. Activity against the drug-sensitive FCR3 strain (IC_{50} = 0.51 nM) was also superior to artemether (IC_{50} = 2.2 nM). To assess the potential for cytotoxicity to host cells, the effect of X206 against the human diploid embryonic cell-line MRC5, revealed an IC_{50} value of 551 nM, indicating good selectivity. In an *in vivo* model of *P. berghei* infection in mice, X206 displayed a 50% effective dose value (ED_{50}) of 0.53 mg kg^{-1} by the subcutaneous administration route, compared with 1.5 mg kg^{-1} for artemether. The acute toxicity of compound **(i)** (LD_{50}, subcutaneous) has been reported previously to be 11 mg kg^{-1}, indicating a narrow therapeutic window; however, its selective and potent antimalarial activity suggests that this compound could be an attractive lead compound for chemical optimization.

1 Otoguro, K. *et al.* (2001) Potent antimalarial activities of polyether antibiotic, X-206. *J. Antibiot.* 54, 658–663



(i)

Novel inhibitors of cytokine production

It is well known that various proinflammatory cytokines are produced in response to an inflammatory stimulus. In particular, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are cytokines that are involved in inflammation, and several inhibitors of the action or production of these proteins have shown significant anti-inflammatory effects. One of the ultimate goals of research in this field is the identification of long-acting, orally bioavailable, small-molecule inhibitors of cytokine production. Recently, researchers at Pfizer (Nagoya, Japan) have identified the pyridine natural products **(ii)** and **(iii)** from the fermentation broth of a basidiomycete, *Marasmiellus* sp. CL21624 [2]. Both compounds displayed potent inhibitory activity towards lipopolysaccharide (LPS)-induced production of IL-1β and TNF-α, although the overall activity of **(iii)** was generally higher. Compound **(ii)** showed IC_{50} values of 0.1 μM and 2.6 μM, for inhibition of IL-1β and TNF-α production, respectively. By contrast, compound **(iii)** displayed IC_{50} values of 0.059 μM and 0.59 μM, for inhibition of IL-1β and TNF-α production, respectively, indicating more potent activity. The effects on production of these cytokines were shown not to be a result of effects on regular protein synthesis by the lack of inhibition of leucine uptake at

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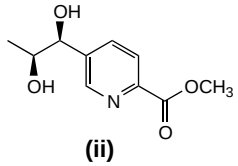
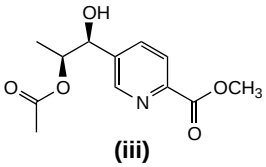
concentrations below 50 μM. These compounds could represent attractive new leads for the discovery of novel small-molecule inhibitors of cytokine production.



(ii)



(iii)

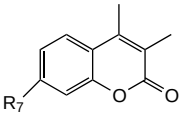
2 Ichikawa, K. *et al.* (2001) Novel cytokine production inhibitors produced by a basidiomycete, *Marasmiellus* sp. *J. Antibiot.* 54, 703–709

Coumarin derivatives as dual inhibitors of acetylcholinesterase and monoamine oxidase

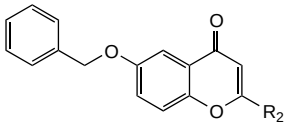
Alzheimer's disease (AD) is neuropathologically characterized by the presence of numerous plaques of amyloid β-peptide (Aβ), neurofibrillary tangles (NFT) and degeneration or atrophy of the basal forebrain cholinergic neurons [3]. The loss of basal forebrain cholinergic cells results in an important reduction in acetylcholine (ACh), which is believed to have an important role in the cognitive impairment associated with AD. Therefore, acetylcholinesterase inhibitors have been seen as a promising approach for the symptomatic treatment of AD. Moreover, the hypothesis has been made that the peripheral binding site of AChE could be involved in Aβ deposition.

Recently, it has been suggested that, among others, monoamine oxidase B (MAO-B) inhibitors could be useful in the treatment of AD. In fact, it was shown that MAO-B activity increases up to threefold in the temporal, parietal and frontal cortex of AD patients, with respect to controls. This increase has also been correlated with the development of Aβ plaques [4]. Because Aβ is the main component of the senile plaques found in AD brains, any compounds that are able to inhibit its aggregation could be regarded as potentially useful in the treatment of the disease. On these bases, Brühlmann and coworkers [5] have now reinvestigated a series of MAO inhibitors, which, in a recent screening [6], proved to also have some inhibitory activity towards AChE and cholinesterase.

The coumarin derivatives tested **(iv–xix)** inhibited MAO-A and MAO-B in the micromolar to low-nanomolar range, with marked MAO-B selectivity. They all showed K_i values for AChE inhibition in the micromolar range (3–100 μM). They are non-competitive inhibitors and are suggested to bind to the peripheral site of AChE. The most potent inhibitor was 7-[3-(chlorobenzyl)oxy]-3,4-dimethylcoumarin [compound **(xi)**; $K_i = 3.40$ μM], which also showed a high MAO-inhibition, accompanied by good A:B selectivity ($pIC_{50} = 5.95$ and 8.48 towards MAO-A and MAO-B, respectively). Insertion of smaller groups (e.g. hydroxy or methoxy) at position 7, resulted in mixed AChE inhibitors. It should also be noted that replacing the coumarin nucleolus by chromone [compounds **(xx)** and **(xxi)**] brought about a significant loss of activity towards both enzymes.



(iv–xix)



(xx) $R_2 = H$
(xxi) $R_2 = CH_3$

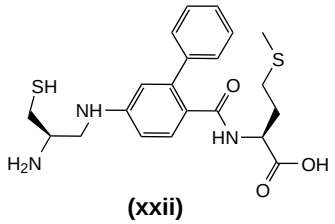
Although further investigations are required, these compounds, which act as dual inhibitors of acetylcholinesterase and MAO, could represent a novel approach to the treatment of AD.

3 Roberson, M.R. *et al.* (1997) Cholinergic activity and amyloid precursor protein metabolism. *Brain Res. Rev.* 25, 50–69
4 Saura, J. *et al.* (1994) Increased monoamine oxidase B activity in plaque-associated astrocytes of Alzheimer brains revealed by quantitative enzyme radioautography. *Neuroscience* 62, 15–30
5 Brühlmann, C. *et al.* (2001) Coumarin derivatives as dual inhibitors of acetylcholinesterases and monoamine oxidase. *J. Med. Chem.* 44, 3195–3198
6 Gnerre, C. *et al.* (2000) Inhibition of monoamine oxidases by functionalized coumarin derivatives: biological activities, QSARs and 3D-QSARs. *J. Med. Chem.* 43, 4747–4758

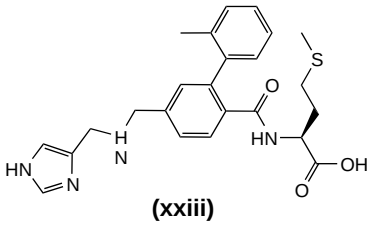
Substituted pyrroles as novel farnesyltransferase inhibitors

Mutated versions of three human *ras* genes are frequently found in many human cancers. This suggests an important role of aberrant Ras function in human tumor growth [7]. The p21 Ras oncogenic products are synthesized as cytosolic proteins, which undergo post-translational modifications for attachment of the normal, as well as mutated, Ras proteins to the membrane [8]. A key step in a series of posttranslational modifications of the oncogenic product, Ras, is farnesylation of the thiol group of the cysteine residue located at the C-terminal CAAX sequence in Ras proteins, which is catalyzed by farnesyltransferase (Ftase) [9]. Farnesyltransferase inhibitors (FTIs) would, therefore, have potential as anticancer agents for tumors in which a *ras* gene is oncogenically mutated.

Numerous FTIs that mimic the tetrapeptide of the Ras C-terminal CAAX motif have been reported, among others, compound **(xxii)** ($IC_{50} = 0.5$ nM) and compound **(xxiii)** ($IC_{50} = 1.4$ nM). Although they are potent, the compounds showed poor cell-membrane

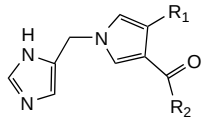


(xxii)



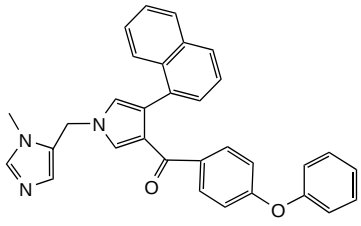
(xxiii)

permeability and poor oral bioavailability [10]. Recently, Lee and coworkers [11] have reported a new series of inhibitors, which lack the carboxylic and the thiol moieties, based on a non-peptidic template. The new compounds are 3-aryl-4-aryloyl-1-(1*H*-imidazol-5-yl)-methylpyrroles **(xxiv–xxxiv)**. SAR studies showed that: (1) the hydrophobic aromatic substituent at C-3 of the pyrrole is crucial to the inhibitory potency of this series; (2) reduction of the ketone led to a 10-times loss in activity, which suggests that the ketone has an important role as both a hydrogen bond acceptor and as a geometric restrictor; and (3) methylation at N-1 of the imidazole ring increased the potency, suggesting that the N-3 imidazole could be involved in binding with the Zn^{2+} of Ftase. The most potent compound was **(xxviii)** (IC_{50} = 4.6 nM).



(xxiv–xxxiv)

R_1 = phenyl, 1-napthyl, 2-napthyl
R_2 = phenyl, substituted-phenyl



(xxviii)

Although further studies are required to ascertain the real importance of these pyrrole derivatives, they represent a new class of FTIs that do not have the problematic thiol and carboxylate groups. Therefore, they could be valuable for the development of farnesyltransferase inhibitors as clinically useful anticancer agents.

7 Casey, P.J. *et al.* (1989) p21Ras is modified by a farnesyl isoprenoid. *Proc. Natl. Acad. Sci. U. S. A.* 86, 8323–8327

8 Der, C.J. and Cox, A.D. (1991) Isoprenoid modification and plasma membrane association: critical factors for ras oncogenicity. *Cancer Cells* 3, 331–340

9 Reiss, Y. *et al.* (1990) Inhibition of purified p21ras farnesyl:protein transferase by Cys-AAX tetrapeptides. *Cell* 62, 81–88

10 Sun, J. *et al.* (1999) Antitumor efficacy of a novel class of non-thiol-containing peptidomimetic inhibitors of farnesyl-transferase and geranylgeranyltransferase I: combination therapy with the cytotoxic agents cisplatin, taxol and gemcitabine. *Cancer Res.* 59, 4919–4926

11 Lee, H. *et al.* (2001) 3-Aryl-4-aryloyl-1-(1*H*-imidazol-5-yl)methylpyrrole, a novel class of farnesyltransferase inhibitors. *Bioorg. Med. Chem. Lett.* 11, 2963–2965

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Drug delivery

A drug-loaded tumor cell system for lung targeting

The current prognosis for a patient with lung cancer is not promising; the eventual death rate is over 90%. Additionally, metastatic cancers often metastasize to the lung, producing further complications. Most anticancer drugs are cytotoxic to healthy tissues, so there is a limited dose that can be administered in a patient's lifetime; this dose is often insufficient to eradicate the cancer. Therefore, a cancer-targeting drug delivery system that is selective for tumor cells versus healthy tissue would be a significant advance. Several approaches have been tried, including liposomes, antibody-linked drug molecules and soluble macromolecule drug conjugates, but each of these methods has drawbacks. There is still a need for a practical tumor-targeting drug delivery system.

There is considerable evidence that metastasis is a nonrandom and organ-specific process. The ease of interaction of tumor cells with the endothelium is thought to be the underlying factor determining the organ preference of metastasis. Successful blood-borne metastasis depends on the ability of a tumor cell to form emboli with other tumor cells and adhere to endothelial cells of the target organ of metastasis. Several adhesion molecules, including integrins, immunoglobulins and selectins, mediate these tumor–host interactions. Clinical findings have also shown that the lung is a major target organ of metastasis, with nearly 40% of all malignancies developing lung metastases, as most blood-borne metastatic cells are filtered out in the pulmonary vesicular system after they are shed into the circulatory system from primary tumors.

Shao and coworkers have recently demonstrated the application of drug-loaded tumor cells (DLTCs) as a drug delivery system for metastatic tumor cell targeting, particularly in the lungs. Two recent papers report the preparation and pharmacokinetics of DLTCs and their application to the treatment of lung cancer in an animal model [1,2]. The group hypothesized that DLTCs in which the tumor cells are functionally dead will retain their membrane surface structure and can be used as an ideal drug carrier, not only for lung-tumor targeting but also for the prevention of metastasis by competing for endothelial metastatic binding sites with the live tumor cells.

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