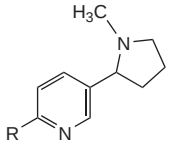
Monitor: molecules and profiles

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.

Molecules

New nicotine derivatives can antagonize its own analgesic action

Nicotine (**i**, R = H) is believed to produce some of its effects through nicotinic acetylcholine (nACh) receptors. Neuronal nicotinic receptors are involved in several physiological processes, including appetite, memory, analgesia, anxiety and other neurological disorders [1–3]. Several side-effects associated with nicotine have led to the search for less toxic nACh agents.



(i-vi)

Glennon and collaborators have reported [4,5] that 6-methylnicotine is more potent than nicotine in *in vivo* assays that are indicative of nicotinic activity, although its affinity is no greater than that of nicotine. In addition, the 6-halogenated analogues of nicotine were found to be more potent than expected on the basis of their affinities.

The same group has now investigated [6] a series of 6-alkylsubstituted analogues of nicotine (compounds **ii–vi**), where the R group ranges from methyl to *n*-pentyl. The compounds were tested in binding experiments as well as in three functional assays: (1) the tail-flick assay (a common test for analgesic properties), (2) spontaneous activity assay in mice, and (3) a substitution in a drug discrimination assay in rats trained to discriminate (–)nicotine from saline vehicle.

Binding data showed that affinity decreases from 6-methylnicotine (**ii**; K_i = 1.8 nM) to 6-*n*-pentylnicotine (**vi**; K_i =72 nM). In the same assay, nicotine showed a K_i value of 1.3 nM. Unexpectedly, in *in vivo* tests (tail-flick and spontaneous activity assays in mice) compound (–)**iii** (R = ethyl, K_i = 5.6 nM) was more potent than nicotine (ED_{50} 2.1 μmol kg^{-1} and 1.4 μmol kg^{-1} versus ED_{50} = 9.9 μmol kg^{-1} and 4.9 μmol kg^{-1} for nicotine, respectively in the two assays), while (–)**iv** (R = *n*.propyl, K_i = 22 nM) was inactive in producing antinociceptive effects up to doses of 70 μmol kg^{-1}. Compounds (–)**v** (R = *n*.butyl, K_i = 21 nM) and **vi** were also inactive in *in vivo* assays.

In further experiments, compound (–)**iv** was found to antagonize the antinociceptive action of 2.5 mg kg^{-1} of (–)nicotine (tail-flick assay; AD_{50} = 4.9 μmol kg^{-1}). By contrast, (–)**iv** did not antagonize either the spontaneous activity or stimulus effects of nicotine at doses up to 35 μmol kg^{-1} and 50 μmol kg^{-1}, respectively.

If multiple subtypes of nACh receptors are involved in the different actions of nicotinic agonists, compound (–)**iv** and other nicotinic competitive antagonists that are able to selectively block different nicotinic effects, could be useful tools in the study of nACh receptor-mediated events.

1 Holladay, M.W. *et al.* (1997) Neuronal nicotinic acetylcholine receptors as targets for drug discovery. *J. Med. Chem.* 40, 4169–4194
2 Glennon, R.A. *et al.* (1999) In *Neuronal Nicotinic Receptors* (Arneric, S.P. and Brioni, J.D., eds), pp 271–284, John Wiley and Sons, New York
3 Glennon, R.A. and Dukat, M. (2000) Central nicotinic receptor ligands and pharmacophores. *Pharm. Acta. Helv. 74*, 103–114
4 Dukat, M. *et al.* (1999) Synthesis, receptor binding and QSAR studies on 6-substituted nicotine derivatives as cholinergic ligands. *Eur. J. Med. Chem.* 34, 31–40
5 Dukat, M. *et al.* (1996) Pyrrolidine-modified and 6-substituted analogs of nicotine: a structure-affinity investigation. *Eur. J. Med. Chem.* 31, 875–888
6 Dukat, M. *et al.* (2002) (–)6-*n*-Propylnicotine antagonizes the antinociceptive effects of (-)nicotine. *Bioorg. Med. Chem. Lett.* 12, 3005–3007

Daniela Barlocco
University of Milan
Viale Abruzzi 42
Milano 20131, Italy
tel: +39 02 5031 7515
fax: +39 02 5031 57565
e-mail: daniela.barlocco@unimi.i

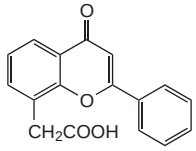
Novel antitumour molecules

New antitumour xanthen-9-one-4-acetic acid analogues

Flavone-8-acetic acid (FAA; compound **i**) is a synthetic flavonoid with an unusual pharmacological profile. It is characterized by low activity against fast-growing tumours, such as leukaemias, but broad activity against slow-growing solid tumours. These slow-growing tumours are

  PII: S1359-6446(02)02552-7

often insensitive to cytotoxic drugs and are without the myelosuppression often observed with cytotoxics. However, its low potency has meant that high doses and long exposure times have been required for a direct cytotoxic effect.

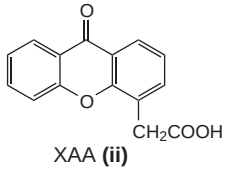


FAA **(i)**

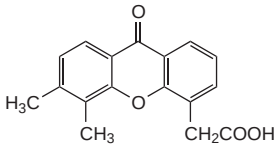
FAA is believed to act, at least in part, as a biological response modifier because it stimulates the activity of natural killer cells and enhances the lytic potential of macrophages in mice. These effects are known to be mediated by the induction of cytokines, such as tumour necrosis factor α (TNF-α) and interferons. Unfortunately, the promising *in vitro* and *in vivo* activity associated with FAA has not been reproduced in clinical trials.

Xanthen-9-one-4-acetic acid (XAA; compound **ii**) and related compounds, such as the 5,6-dimethyl analogue (DMXAA; compound **iii**), are structural analogues of FAA that show higher potency and the ability to induce cytokines, such as TNF-α in human cells; DMXAA has now completed Phase I clinical trials.

Gobbi and co-workers (University of Bologna, http://www.unibo.it; University of Padova, http://www.unipd.it) have reported the synthesis and antitumour evaluation of a series of XAA analogues in which the 5 and 6 substituents are included in cyclic structures, or bear a large lipophilic substituent in position 6 [1].

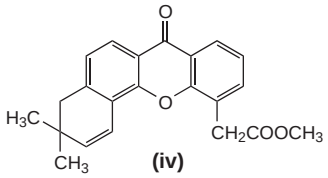


XAA **(ii)**



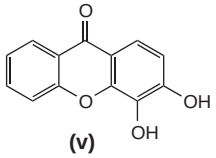
DMXAA **(iii)**

When the direct *in vitro* toxicity in four human cancer cell lines (LoVo S and Lovo R from human colon adenocarcinoma and 2008 and C13* from ovarian adenocarcinoma) was evaluated, the new analogues generally showed low direct toxicity. More significantly, the new compounds enhanced the lytic properties of both murine peritoneal macrophages and human monocytes, as well as reference compounds XAA and DMXAA. Certain new compounds (e.g. compound **iv**) showed higher activity than the previous lead compound on human monocytes and could induce TNF-α production in human immune cells.

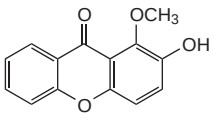


(iv)

In related work, Pedro and co-workers (Fitoquímica e Farmacologia da Universidade do Porto, http://www.up.pt/conhecaup/facinst/ffup/ffup.htm) have reported the synthesis of 27 oxygenated xanthones and assessed their ability *in vitro* to inhibit the growth of three human cancer cell lines (breast MCF-7, renal TK-10 and melanoma UACC-62) [2]. The effects of these new compounds on the proliferation of human T-lymphocytes were also assessed. In general, differences in potency, in terms of cell line and T-lymphocyte inhibitory activity, can be ascribed to the nature and positions of substituents on the xanthone nucleus. For example, dihydroxylation at all positions was associated with more potent inhibitory activity in all three cell lines compared to the corresponding dimethoxy analogues. The dioxygenated derivatives **v** and **vi** showed the most potent inhibitory activity in the human T-lymphocyte assay (IC_{50} values of 12 μM in each case).



(v)

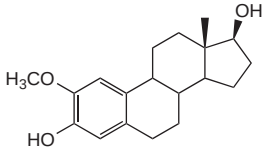


(vi)

1 Gobbi, S. *et al.* (2002) Synthesis and antitumor activity of new derivatives of xanthen-9-one-4-acetic acid. *J. Med. Chem.* 45, 4931–4939
2 Pedro, M. *et al.* (2002) Xanthones as inhibitors of growth of human cancer cell lines and their effects on the proliferation of human lymphocytes *in vitro*. *Bioorg. Med. Chem.* 10, 3725–3730

Inhibitors of tubulin polymerization

Several interesting antitumour properties of the endogenous human metabolite 2-methoxyestradiol (**vii**), including the inhibition of tumour growth, metastasis and angiogenesis *in vivo*, have generated interest in this agent as a potential clinical anticancer agent. It has a low affinity for the estrogen receptor, and evidence suggests that its cytotoxic activity might result from inhibition of tubulin polymerization through binding to the colchicine binding site. The microtubule system of eukaryotic cells is an important anticancer drug target, and antimitotic compounds that interfere with microtubule-tubulin equilibria have proven useful agents in the clinic, for example, paclitaxel from the bark of the Pacific yew tree and the epothilones isolated from the bacterium *Sorangium cellulosum*. The low bioavailability of 2-methoxyestradiol following oral administration in mice has led to further work in the design and synthesis of analogues with enhanced potency and metabolic stability.

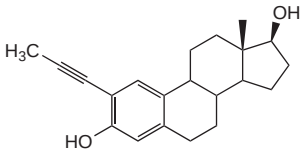


(vii)

Cushman and co-workers (Purdue University School of Pharmacy and Pharmacal Sciences, http://www.pharmacy.purdue.edu) have reported the synthesis of a new series of estradiol derivatives

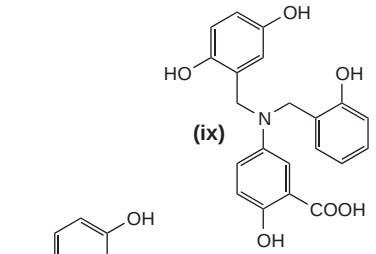
  PII: S1359-6446(02)02551-5

bearing various substituents at the 2-position, to further probe the structural requirements for antitubulin activity and cytotoxicity [3]. Several of these new derivatives were found to inhibit tubulin polymerization in the low micromolar range [e.g. 2-(1'-propynyl)estradiol compound **viii**, IC_{50} for inhibition of tubulin polymerization = 5 μM], although remaining compounds were inactive up to concentrations of 40 μM. Generally, the new compounds were cytotoxic in the low micromolar range against a range of human cancer cell lines *in vitro*, and cytotoxicity correlated with potency of tubulin polymerization inhibition. Compound **viii** also displayed significant activity in the *in vivo* hollow fiber model where polyvinylidene fluoride hollow fibers containing various cancer cell cultures were implanted intraperitoneally (IP) or subcutaneously (SC) into mice and the compound administered by the intraperitoneal (IP) route.

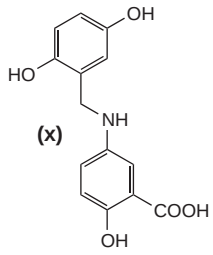


(viii)

Interest in the *Streptomyces griseolavendus* metabolite lavendustin A (**ix**) originally arose from its epidermal growth factor (EGF) receptor tyrosine kinase inhibitory activity, a kinase whose activity is elevated in several cancers. Further analysis of this agent led to the identification of compound **x** as the biologically active fragment. Although lavendustin A was found to be inactive as a PTK inhibitor in cellular systems, the methyl ester of **ix** inhibited PTK activity and internalization of the EGF receptor in cell culture. The synthesis of derivatives of general structure **xi** was also found to inhibit PTK activity in cellular systems. Further analysis of compounds of general structure **xi** suggested that the cytotoxicities could be caused by inhibition of tubulin polymerization, and IC_{50} values for both inhibition of tubulin polymerization and for cytotoxicity were found to



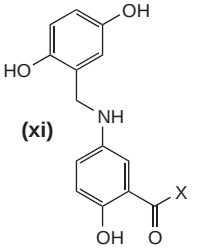
(ix)



(x)

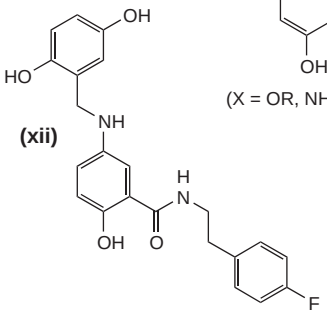
be relatively close. The most cytotoxic compound from series **xi** was found to be the lavendustin analogue **xii**. This, and related compounds, can exist in different conformations (represented by isoindolone **xiii** or oxazinedione **xiv**) resulting from rotation around the bond connecting the amide carbonyl to the aromatic ring.

Mu and co-workers from the Cushman group have reported the synthesis and antitumour evaluation of conformationally restricted analogues of lavendustin A as cytotoxic inhibitors of tubulin polymerization [4]. Surprisingly, however, the cell line cytotoxicity and tubulin polymerization inhibition results indicated little effect of conformational restriction on biological activity. The authors speculate that the hydroquinone ring of lavendustin A could be a more important determinant of biological activity than the structure surrounding the aniline ring.

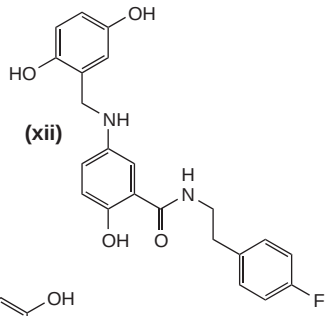


(xi)

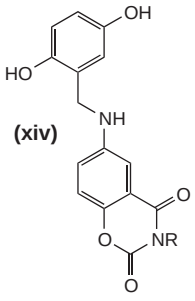
(X = OR, NHOR, NHR)



(xii)



(xii)



(xiv)

3 Cushman, M. *et al.* (2002) The effect of exchanging various substituents at the 2-position of 2-methoxyestradiol on cytotoxicity in human cancer cell cultures and inhibition of tubulin polymerization. *J. Med. Chem.* 45, 4748–4754

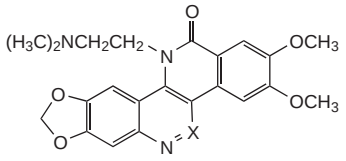
4 Mu, F. *et al.* (2002) Synthesis and investigation of conformationally restricted analogues of lavendustin A as cytotoxic inhibitors of tubulin polymerization. *J. Med. Chem.* 45, 4774–4785

Potent topoisomerase targeting antitumour agents

DNA topoisomerases regulate DNA topology through the breaking and rejoining of DNA strands, and are crucial to both replication and transcription. The topoisomerases are divided into two groups, I and II, depending on whether the initial mechanism of action involves single- or double-strand DNA breaks. Agents that can stabilize the cleavable complex formed between the enzyme and DNA have proven effective as anticancer agents and several of such compounds are in clinical use, for example, the topoisomerase I-targeting camptothecin analogues topotecan (Hycamptin®) and irinotecan (Camptosar®).

Both these agents contain a γ-lactone ring prone to hydrolysis to an inactive derivative; therefore, because both are substrates for drug efflux transporters, attention has recently been directed to

new topoisomerase targeting agents devoid of these drawbacks. Ruchelman and co-workers (Rutgers University, http://www.rci.rutgers.edu/~layla/Faculty/LaVoie.htm; The Cancer Institute of New Jersey, http://cinj.umdnj.edu; The University of Medicine and Dentistry of New Jersey, http://www.umdnj.edu) have reported the synthesis of several 5,12-diazachrysen-6-ones and 5,6,11-triazachrysen-12-ones with varied substituents at the 5- or 12-position respectively [5].



(xv) (X = CH)
(xvi) (X = N)

The new series were based on the structurally related benzo[*i*]phenanthridine and dibenzo[*c,h*]cinnoline derivatives that were found to possess significant topoisomerase I-targeting activity and cytotoxicity in human cancer cell lines; however, difficulties relating to poor water solubility were encountered in developing suitable formulations for assessment of their *in vivo* efficacy. The new compounds were evaluated for their ability to stabilize the cleavable complex formed between topoisomerase I and DNA. Two analogues with potent topoisomerase I-targeting activity (**xv** and **xvi**) exhibited potent activity (IC_{50} of < 2 nM) against RPMI8402 (human lymphoblast tumour cell line). Compound **xv** was also found to be active *in vivo* in the human MDA-MB-435 tumour xenograft athymic nude mice model.

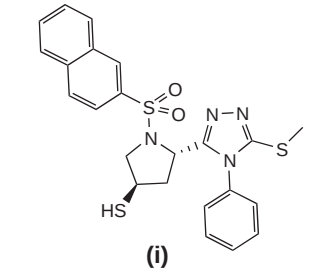
5 Ruchelman, A.L. *et al.* (2002) Diaza- and triazachrysenes: potent topoisomerase-targeting agents with exceptional antitumor activity against the human tumor xenograft, MDA-MB-435. *Biorg. Med. Chem. Lett.* 12, 3333–3336

Andrew D. Westwell
School of Pharmaceutical Sciences
University of Nottingham
Nottingham, UK NG7 2RD
tel: +44 115 951 3419
fax: +44 115 951 3412
e-mail: andrew.westwell@nottingham.ac.uk

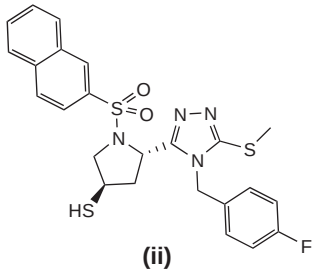
Combinatorial chemistry

Endothelin Converting Enzyme inhibitors

Endothelin-1 (ET-1), a 21 amino acid peptide, is a potent, long-acting vasoconstrictor. High plasma levels are found in many clinical conditions, such as congestive heart failure, subarachnoid haemorrhage and pulmonary hypertension. Endothelin is produced from its biologically inactive precursor big-ET by the Zn-endopeptidase, endothelin converting enzyme (ECE). The current understanding is that the inhibition of ECE-1 might enable the specific blockage of the whole ET system, and is therefore an attractive therapeutic approach. Kitas and co-workers (Hoffmann–La Roche, http://www.roche.com) have attempted to improve the potency of their lead compound (**i**) and to elucidate SAR by modifying three sites on it [1]. A small library was synthesized on solid phase in an attempt to generate potent ECE inhibitors. The library compounds were evaluated for their inhibition of hECE-1. Compound **ii** was one of the most potent found, with an IC_{50} value of 150 nM. This work has produced potent ECE inhibitors, and holds promise for further optimization.



(i)

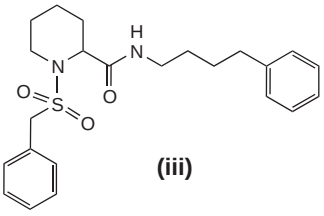


(ii)

1 Kitas, E.A. *et al.* (2002) Synthesis of triazole-tethered pyrrolidine libraries: novel ECE inhibitors. *Bioorg. Med. Chem. Lett.* 12, 1727–1730

FKBP12 inhibitors

Immunophilins are enzymes that possess peptidyl-prolyl isomerase (PPIase) activity, and bind the immunosupressant drugs FK506, cyclosporin A and rapamycin. Small molecule ligands for the immunophilin FKBP12 show promise as a powerful new strategy for treating degenerative disorders of the nervous system. These compounds possess potent neurotropic actions *in vitro* and *in vivo*, and promote structural and functional recovery in animal models of neurodegenerative disease. Studies have been undertaken to explore the therapeutic use of various classes of FKBP12 ligands using combinatorial chemistry techniques [2]. A library of 120 compounds was synthesized on Argogel-polystyrene solid phase resin with acylsulfonamide linker. The library compounds were screened for inhibition of rotamase activity of FKBP12 using the peptide *N*-succinyl Ala-Leu-Pro-Phe *p*-nitroanilide (Bachem) as substrate, and a *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioning of dopaminergic neurons in mice, which is used as an animal model of Parkinson's disease. Compound **iii** was one of the most potent isolated, with a K_i value of 1100 nM. This work has provided novel, potent leads worthy of further investigation.



(iii)

2 Wu, Y-Q. *et al.* (2002) Solid-phase synthesis of FKBP12 inhibitors: *N*- sulfonyl and *N*-carbamoylprolyl/pipecolyl amides. *Bioorg. Med. Chem. Lett.* 12, 1429–1433

Paul Edwards
Discovery Chemistry
Pfizer Global Research and Development
Sandwich
Kent, UK
fax: +44 1304 643 555
e-mail: paul_edwards@sandwich.pfizer.com

 1359-6446/02/$ – see front matter PII: S1359-6446(02)02550-3