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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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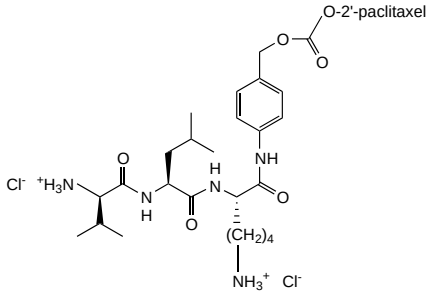
Molecules

Paclitaxel prodrugs: selective activation by the tumor-associated protease plasmin

The lack of selectivity for tumor cells of chemotherapeutic agents such as paclitaxel (Taxol) is still a serious drawback in conventional cancer chemotherapy. The use of tumor-associated enzymes capable of selective conversion of non-toxic prodrugs into the active agent in a site-selective manner is an attractive strategy. One such enzyme is the serine protease plasmin, which is known to play a key role in tumor invasion and metastasis.



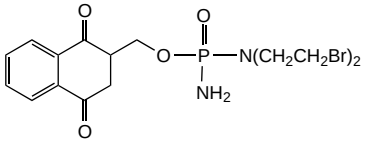
(i)

Scheeren and co-workers at the University of Nijmegen (Netherlands) have designed and synthesized a series of stable 2′-carbamate- and 2′-carbonate-linked prodrugs of paclitaxel that show, on average, a decrease in cytotoxicity of more than 8000-fold in comparison to the parent compound in human tumor cell lines (e.g. MCF-7, EVSA-T and WIDR)[1]. Two prodrugs undergo selective hydrolysis by human plasmin and prodrug **(i)** was selected as the most promising for further study on the basis of enzyme hydrolysis and spacer elimination rates.

1 De Groot, F.M.H. *et al.* (2000) Synthesis and biological evaluation of 2′-carbamate-linked and 2′-carbonate-linked prodrugs of paclitaxel: selective activation by the tumor-associated protease plasmin. *J. Med. Chem.* 43, 3093–3102

Novel quinone phosphorodiamidate prodrugs targeted to DT-diaphorase

DT-diaphorase, a cytosolic flavin adenine dinucleotide (FAD)-containing enzyme, which catalyses the two-electron reduction of quinones, has become an attractive target for the design of bioreductively activated prodrugs because the enzyme is known to be overexpressed in a number of solid tumors. Borch and co-workers at Purdue University (Indiana, USA) have synthesized a series of naphthoquinone and benzimidazolequinone phosphorodiamidates that were all excellent substrates for human DT-diaphorase[2].



(ii)

The naphthoquinones, e.g. **(ii)** were found to be potent cytotoxic agents, undergoing facile activation and expulsion of the cytotoxic phosphorodiamidate either by reduction or via reaction with glutathione.

2 Borch, R.F. *et al.* (2000) Development of novel quinone phosphorodiamidate prodrugs targeted to DT-diaphorase. *J. Med. Chem.* 43, 3157–3167

Human hdm2/p53 interaction antagonists

The hdm2 protein is known to have an important role in the regulation of tumor suppressor p53 expression through binding to the transactivation domain of p53 and downregulating its ability to activate transcription. p53 then activates the expression of the hdm2 gene in an autoregulatory negative feedback loop. In addition, hdm2 targets p53 for degradation by the ubiquitin pathway. Disruption of the p53/hdm2 protein–protein interaction in tumor cells should, therefore, lead to accumulation of p53 and activation of p53 responsive genes – an attractive strategy for cancer therapy. A report from the Oncology Research and Core Technologies Group at Novartis (Basel, Switzerland), details the use of structural information to increase the hdm2 binding affinity of short peptide motifs such as **(iii)** (IC_{50} in wt p53/glutathione *S*-transferase (GST)-hdm2 inhibition assay = 5 nM) derived from the

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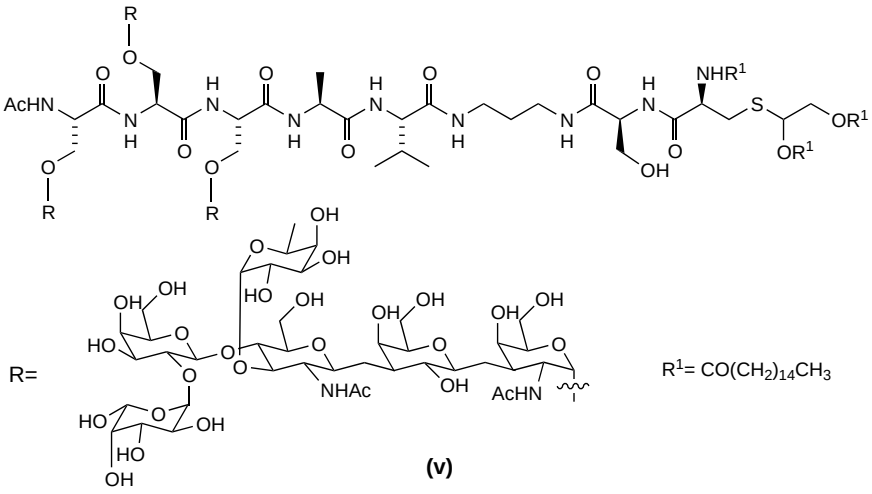
N-terminal domain of the human wild type (wt) p53 (Ref. 3). The new interactions identified and confirmed experimentally could be directly applied to the optimization of nonpeptidic leads or in the *de novo* design of antagonists of the p53/hdm2 interaction.

Ac-Phe[19]-Met-Aib-Pmp-6-Cl-Trp-Glu-
Ac_3c-Leu[26]-NH_2

(iii)

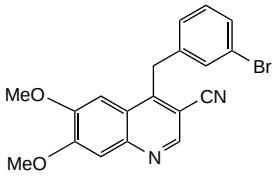
3 Garcia-Echeverria, C. *et al.* (2000) Discovery of potent antagonists of the interaction between human double minute 2 and tumor suppressor p53. *J. Med. Chem.* 43, 3205–3208



(v)

New epidermal growth factor receptor kinase inhibitors

Wissner and co-workers at Wyeth-Ayerst Research (New York, USA), have reported the synthesis and evaluation of a series of 4-anilino-6,7-dialkoxyquinoline-3-carbonitriles **(iv)** as inhibitors of the tyrosine kinase epidermal growth factor receptor (EGF-R), which is overexpressed in numerous tumors and is correlated with poor prognosis in diseases such as brain, lung and bladder cancer[4]. This new series bear a biosteric relationship to the familiar 4-anilino-6,7-dialkoxyquinazoline EGF-R inhibitors currently undergoing clinical evaluation and were found to have EGF-R inhibitory activity comparable to the quinazoline inhibitors.



(iv)

4 Wissner, A. *et al.* (2000) 4-Anilino-6,7dialkoxyquinoline-3-carbonitrile inhibitors of epidermal growth factor receptor kinase and their biosteric relationship to the 4-anilino-6,7-dialkoxyquinazoline inhibitors. *J. Med. Chem.* 43, 3244–3256

The design and synthesis of Le^y-bearing glycopeptides

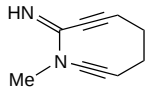
The Danishefsky group at the Sloan-Kettering Institute for Cancer Research (NY, USA) continue to pioneer the development of totally synthetic molecular vaccine candidates[5]. These could, in principle, provide 'circulatory immunosurveillance' as a non-toxic means of combating cancer at the stage of disease progression where few alternative therapies are available. Tumor-associated cell surface carbohydrates e.g. $Lewis^y$ (Le^y) are of particular interest in this regard as they are often overexpressed in tumor cells and could therefore be accessible to binding by circulating antibodies. The vaccine candidates e.g. **(v)** comprised a peptide backbone consisting of two regions, a glycodomain, AcNH-SSS-, and a nonglycosylated sequence, -AVAV-. The glycopeptide was conjugated, via an additional spacer, to the lipid carrier PamCysSer. Although full evaluation of immunostimulatory capacity is ongoing, some remarkable observations have already been seen in murine preclinical models. Clustering of the glycodomain was found to be crucial for anti-Le^y antibody production. For example, **(v)** was found to elicit an immune response and the antibodies that were stimulated recognized both Le^y-ceramide and Le^y-mucin glycoproteins.

5 Danishefsky, S.J. *et al.* (2000) Design and synthesis of Le^y-bearing glycopeptides that mimic cell surface Le^y Mucin glycoprotein architecture *J. Amer. Chem. Soc.* 122, 7273–7279

Computer design of enediyne warheads

The enediyne family of antibiotics are known to possess potent cytotoxicity and have been the subject of great interest as potential anticancer drugs. To be therapeutically active, members of this family, such as calicheamicin, must be composed of: (1) a delivery system (polysaccharide), which helps the enediyne to dock into the DNA minor groove; (2) a trigger device consisting of a methyl trisulfide group and a conjugated cyclohexenone; and finally (3) an antitumor warhead, which forms a biradical species upon Bergman cyclization. An extensive theoretical evaluation of thirteen heteroenediyne warheads was undertaken by Kraka and Cremer at the University of Göteborg (Göteborg, Sweden)[6]. Seven criteria were developed and discussed that would lead to the design of a new enediyne anticancer drug that should have low toxicity but high biological selectivity and activity against tumor cells. These criteria concerned issues such as the stability of the species involved in the reaction of an enediyne, the biradical character and H-abstraction ability of the intermediate biradical Bergman product, and the basicity of the enediyne and its associated biradical. On the basis of calculations performed using these criteria, it was proposed that an N,C-dialkynyl aldimine **(vi)**

incorporated into a cyclodecaene ring was the best candidate for a new enediyne anticancer drug.



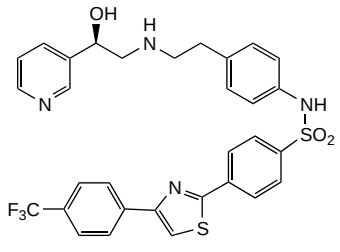
(vi)

6 Kraka, E. and Cremer, D. (2000) Computer design of anticancer drugs. A new enediyne warhead. *J. Amer. Chem. Soc.* 122, 8245–8264

Orally bioavailable β_3-adrenergic receptor agonist as an anti-obesity agent

Obesity is a growing problem in the western world and is closely linked to type II diabetes, coronary heart disease and hypertension. Increasing energy expenditure is one approach to controlling percentage body fat and it has been observed that stimulation of β_3-adrenergic receptors (β_3 AR) located on adipocytes lead to lipolysis and upregulation of uncoupling protein UCP-1, producing an enhancement of metabolic rate.

A group at Merck (Rahway NJ, USA) has improved upon the pharmacokinetic properties of their previously disclosed series of pyridylethanolamine derivatives as potent and selective β_3-AR agonists[7]. Compound **(vii)** has been chosen for clinical evaluation. It is a full agonist with a potency of 35 nM and exhibits an oral bioavailability of 38% and 17% in dogs and rats, respectively. It has a prolonged half-life ($t_{1/2}$ >8 h) in all species tested, displays poor CNS penetration and a good therapeutic index for hyperglycerolaemia over cardiovascular effects.



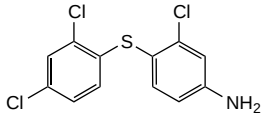
(vii)

7 Mathvink, R.J. *et al.* (2000) Discovery of a potent, orally bioavailable β_3 adrenergic receptor agonist, (*R*)-*N*-[4-[2[[2-hydroxy-2-(3-pyridinyl)ethyl]amino]phenyl]-4-[4-[4-(trifluoromethyl)phenyl]thiazol-2-yl]benzenesulphonamide. *J. Med. Chem.* 43, 3832–3836

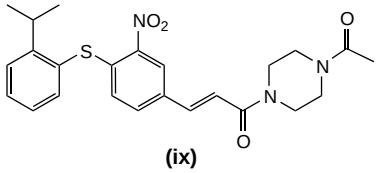
Novel *p*-arylthio cinnamides as inhibitors of the early phase of inflammation

The recruitment of leukocytes to an area of tissue damage or infection is a key event in the inflammatory process. Interactions between proteins on the cell surface of the endothelium and leukocyte lead to cell adhesion and, ultimately, migration of the leukocyte to the inflamed tissue. One of these interactions is between the integrin, the leukocyte function associated antigen-1 (LFA-1) and the intercellular adhesion molecule ICAM-1. An agent that blocks this association could modulate the early steps in the immune response.

A group at Abbott (Abbott Park IL, USA) identified the screening hit **(viii)** with an IC_{50} of 1.7 μM (Ref. 8). Careful pharmacophoric analysis of other screening hits identified an adjacent binding site. By choosing an appropriate linker, both binding pockets were accessed, facilitating a substantial increase in potency and eventually giving rise to compound **(ix)**. This molecule inhibits LFA-1–ICAM-1 binding with an IC_{50} of 44 nM and is also potent in an LFA-1 mediated cell adhesion assay with an IC_{50} of 35 nM. Opimization of the pharmacokinetic properties of this series will help to validate this target.



(viii)



(ix)

8 Liu, K. *et al.* (2000) Discovery of novel *p*-arylthio cinnamides as antagonists of leukocyte function-associated antigen-1/intracellular adhesion molecule-1 interaction. 1. Identification of an additional binding pocket based on an anilino diaryl sulfide lead. *J. Med. Chem.* 43, 4025–4040

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Drug targeting

Antisense radiopharmaceuticals – agents for imaging gene expression

Gene expression *in vivo* can be imaged either indirectly or directly. In the indirect approach, a small-molecule radiopharmaceutical is administered and trapped in a target organ owing to the enzymatic activity of a specific gene product. The indirect approaches to imaging gene expression *in vivo* require pre-existing knowledge of the function of the gene. However, the vast majority of disease-causing genes that are being identified with disease-specific genomics programs, are genes of unknown function. This is illustrated by the Brain Tumor Cancer Genome Anatomy Project (BT-CGAP) database (http://www2.ncbi.nlm.nih.gov/CGAP/hTGI/). Review of this database shows that a total of 13 985 expressed genes have been detected in human brain cancer. Of these, 1095 genes are unique to human brain cancer, and of these 1095 unique genes, only 10 are genes of known function. Therefore, >99% of the expressed genes that are unique to human brain cancer are genes of unknown function! Such information suggests that indirect imaging of pathological gene

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