# Monitor

***Monitor*** provides an insight into the latest developments in the pharmaceutical and biotechnology industries. ***Chemistry*** examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while ***Profiles*** provides commentaries on promising lines of research, new molecular targets and technologies. ***Biology*** reports on new significant breakthroughs in the field of biology and their relevance to drug discovery. ***Business*** reports on the latest patents and collaborations, and ***People*** provides information on the most recent personnel changes within the drug discovery industry.

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# Chemistry

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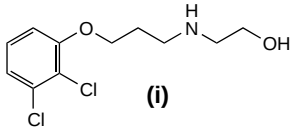
## Anti-cancer molecules

### Selective induction of apoptosis in tumour cells

Resistance to apoptosis represents one of the six hallmarks of cancer [1], and the (indirect) induction of apoptosis in cancer cells is a common theme underpinning the activity of anticancer agents used in the clinic. Unfortunately, however, there are several limiting factors in anticancer therapy that cause treatment failure or discontinuation of treatment. Among these factors, the development of resistance to treatment, and dose-limiting side effects resulting from a lack of discriminatory activity between cancerous cells and those of normal tissue, are extremely prevalent.

As part of efforts towards the development of more selective antitumour agents, many research laboratories are increasingly involved in the search for agents that induce apoptosis specifically in cancer cells while sparing normal tissue. Although the era of efficacious antitumour therapeutic agents with wide clinical applicability largely devoid of side effects is still some way from reality, encouraging new drugs (such as Gleevec [2]) have emerged in recent years to suggest that this vision should be achievable in the medium term. Two recent reports [3,4] detailing agents that are able to induce apoptosis selectively in a range of tumour cell lines further illustrate this approach.

Wu and co-workers from the University of Texas M.D. Anderson Cancer Center and Graduate School of Biomedical Sciences (http://www.mdanderson.org) have described the identification of 2-[[3-(2,3-dichlorophenoxy)propyl]amino]ethanol (2,3-DCPE; compound **i**) from a purchased chemical library, which was found to induce apoptosis more effectively in various cancer cell lines than in normal human fibroblasts *in vitro* [3].

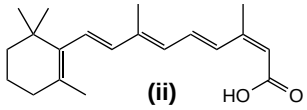



**(i)**

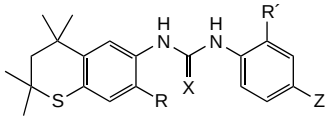
$IC_{50}$ values in the low micromolar range were found for human colon cancer cell lines LoVo and DLD-1, and for human lung cancer cell lines H1299 and A549 (0.89, 1.95, 2.24 and 2.69 μM, respectively); the cell viability assay for normal human fibroblasts gave an $IC_{50}$ value of 12.6 μM. 2,3-DCPE was revealed to cause cancer cell specific cleavage of the apoptotic pathway proteins caspase-8, caspase-3, caspase-9 and poly(ADP-ribose) polymerase, and to cause release of cytochrome c. In addition, 2,3-DCPE attenuated the protein level of the cell survival mitochondrial membrane protein Bcl-XL. Further preclinical drug development data on this new agent are anticipated.

Synthetic retinoids are known to regulate growth, differentiation and apoptosis through poorly understood mechanisms that can be dependent or independent of their ability to bind and activate nuclear retinoic acid receptors. Several retinoids, such as 13-cis-retinoic acid **(ii)**, possess striking antitumour activity; however, application in the clinic has been limited by dose-limiting toxic side effects. Liu and co-workers from Oklahoma State University (http://www.okstate.edu) have now reported the first examples of sulfur-containing heteroarotinoids **(iii)** that induce apoptosis and reactive oxygen species with discrimination between cancer and normal cell lines.

Inhibitory activity in a panel of ovarian cancer cell lines (e.g. Caov-3, OVCAR-3 and SK-OV-3) was compared to the well-known retinoid 4-HPR **(iv)**, which has demonstrated chemopreventative activity

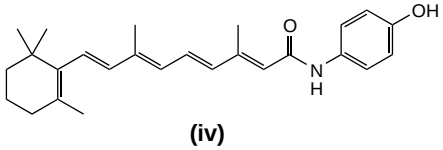



**(ii)**




**(iii)**

X=S,O; Z=$CO_2Et$, $NO_2$, $SO_2NH_2$
R,R'=H, $CH_3$




**(iv)**

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against ovarian cancer in clinical trials. The heteroarotinoids inhibited the growth of all cancer cell lines (low micromolar $IC_{50}$ values), with weak activity against normal and benign cells, when comparing an ovarian cancer cell line (OVCAR-3), a borderline ovarian tumour (O1), a benign ovarian cyst (O3) and normal endometrial cells. Growth inhibition was associated with cell loss (apoptosis) and generation of reactive oxygen species, and was found to be independent of retinoic acid receptor activation. Compound **iiia** (X=S, Z=NO2, R,R'=H) is currently in preclinical development for cancer prevention and treatment (National Cancer Institute; http://www.nci.nih.gov).


1 Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. *Cell* 100, 57–70
2 Capdeville, R. *et al.* (2002) Glivec (STI571, imatinib), a rationally developed targeted anticancer drug. *Nat. Rev. Drug Discov.* 1, 493–502
3 Wu, S. *et al.* (2004) Induction of apoptosis and down-regulation of Bcl-XL in cancer cells by a novel small molecule, 2-[[3-(2,3-dichlorophenoxy)propyl]amino]ethanol. *Cancer Res.* 64, 1110–1113
4 Liu, S. *et al.* (2004) Synthesis of flexible sulfur-containing heteroarotinoids that induce apoptosis and reactive oxygen species with discrimination between malignant and benign cells. *J. Med. Chem.* 47, 999–1007


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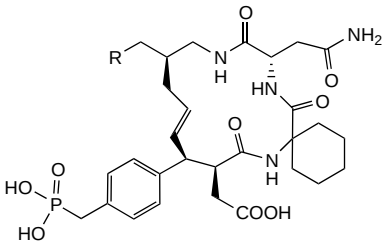
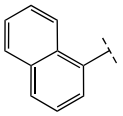
### Ultrapotent Grb-2 SH2 domain-binding ligands

It is known that phosphorylation at particular phosphotyrosine (pTyr) residues serves as an on–off switch for the Src homology 2 (SH2) domain of proteins. This non-catalytic domain recognizes short peptide motifs bearing pTyr; the binding to pTyr sites can thus affect SH2-containing proteins in multiple ways. The growth factor receptor bound protein 2 (Grb-2) is an SH2 domain-containing docking module that represents an attractive target for anticancer therapeutic intervention.

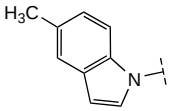
On the basis of the preferential binding of Grb-2 SH2 domain to pTyr-X-N containing sequences in a bend conformation, a series of macrocyclic peptide mimetics carrying a hydrolitically stable phosphonomethylphenylalanine instead of the pTyr residue was prepared. Thus, derivative **va** was found to have high



**(va)** R =



**(vb)** R =



Grb-2 SH2 domain binding potency in both extracellular ($IC_{50}$ = 2 nM) and whole cell assays ($IC_{50}$ ≥1 μM) [5] despite the presence of anionic functionalities, which often represent a limitation for cell membrane transit. In particular, the presence of the acidic functionality at the pTyr mimetic α position is essential to obtain high potency in whole-cell assays.

Then, on the basis of molecular modeling studies, **vb** was synthesized, with the aim to enhance the hydrophobic interactions with the SH2 domain. The resultant compound showed a *K*d value of 92.7 pM; thus, **vb** is significantly more potent that **va** (**va**: *K*d = 0.91 nM) and is the highest affinity agent yet reported for a synthetic Grb-2 SH2 domain-binding ligand [6]. It is also significantly more active than compound **va** in cellular assays and is able to elicit antimitogenic effects in growth-factor-driven breast cancer cells at noncytotoxic submicromolar concentrations [6]. These results indicate a high potentiality of this class of signal-transduction altering compounds as therapeutic agents.


5 Wei, C-Q. *et al.* (2003) Macrocyclization in the design of Grb-2 SH2 domain-binding ligands exhibiting high potency in whole cell systems. *J. Med. Chem.* 46, 244–254
6 Shi, Z-D. *et al.* (2004) Synthesis of a 5-methylindolyl-containig macrocycle that displays ultrapotent Grb2 SH2 domain-binding affinity. *J. Med. Chem.* 47, 788–791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# Biology

## Microbiology

### Respiratory chain inhibition and pentamidine: a new drug combination against leishmaniasis?

Chemotherapy constitutes the main tool for control of leishmaniasis, but antileishmanial drugs are rare, expensive and toxic, and their mechanisms of action are elusive. Mehta and Shaha now show that concomitent administration of respiratory chain complex II inhibitors with pentamidine increases the drug cytotoxicity against promastigotes of *Leishmania donovani* [1].

The authors first observed that inhibition of the respiratory complexes II and III by TTFA and antimycin A, respectively, resulted in the dissipation of the mitochodrial membrane potential. On the contrary, rotenone, an inhibitor of respiratory complex I induced a mitochondrial hyperpolarization. These changes in Dym caused, as expected, a reduction of the cell viability, but more interestingly this loss was attributed to apoptosis as cells showed DNA fragmentation and externalization of phosphatidyl serine.

In a second step the authors tried to understand by which mechanism the Dym alteration occured. They observed that inhibition of complexes II and III resulted in the generation of reactive oxygen species (ROS) and in an increase of the intracellular calcium concentration. Because ROS increase was not blocked by calcium sequestration (EGTA) and an ROS scavenger inhibited the increase in intracellular calcium, the authors concluded that ROS generation was responsible for the calcium increase.

Pentamidine was known to accumulate in the mitochodria causing a loss of Dym . The combined use of pentamidine and TTFA led to a fourfold increase in intracellular calcium and an increase in cell


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