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.**

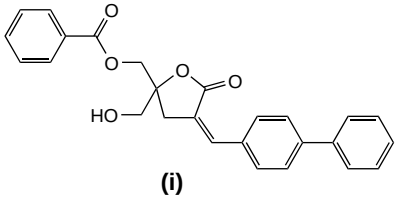
Chemistry

Combinatorial chemistry

Protein kinase C isozymes are potential targets for cancer therapy

Protein kinase C (PKC) phosphorylates a variety of cellular proteins and has a pivotal role in signal transduction mechanisms, making this enzyme an important therapeutic target for cancer and other diseases. The regulatory domains of the PKC isozymes, which are a family of related serine and threonine kinases, comprise several copies of cysteine-rich motifs (C1 domains) that are approximately 50 amino acids in length. The C1 domains of the conventional (α, β1, β2 and γ) and novel (δ, ε, η and θ) subtypes of PKC bind phorbol ester tumour promoters and the second messenger diacylglycerol (DAG) [1]; it is the binding of these cofactors that induces the translocation and activation of these classes of PKC. The atypical isoforms of PKC (ζ and λ) contain a single non-functional C1 domain that does not bind either phorbol esters or DAG. There are also other families of proteins that contain C1 domains that are similarly responsive to phorbol esters and DAG. Although the kinase domains of the PKCμ family superficially resemble the kinase domains of PKC, there are only low levels of homology between these domains, which also exhibit differing selectivity.

Among the kinase and non-kinase C1 domains, there are important amino acid variations in the β-loops, which conform the area of the domain through interaction with the membrane. An extensive exploration of the chemical diversity of PKC substrates could lead to the discovery of specific C1-domain ligands for each class of these receptors [2]. To accomplish this goal, Duan *et al.* [2] developed a solid-phase method for the synthesis of a library of nine compounds to validate this strategy. Each compound in this array was purified and PKC-binding affinities for the PKCα-isozyme were compared with those obtained for the crude products. Because phorbol esters and DAG-lactones compete for the same site on the enzyme (the C1 domain), a convenient binding assay was the measurement of the level of displacement of [20-^{3}H]phorbol-12,13-dibutyrate (bound to recombinant isozyme PKCα) by DAG-lactone in the presence of phosphatidylserine (expressed as K_i values). This study revealed a less than fourfold variation in K_i between purified and crude samples.



(i)

The mini-library of nine compounds was synthesized using MacroKan® technology (IRORI; http://www.irori.com). Synthesis was performed using a 3,4-dihydro-2*H*-pyran resin. One of the most potent compounds discovered was **i**, which had a K_i on purified material of 14 nM. Accordingly, this research provides valuable tool compounds with high-affinity for PKC and further work in this area is warranted.

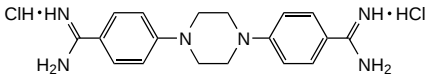
1 Ron, D. and Kazanietz, M.G. (1999) New insights into the regulation of protein kinase C and novel phorbol ester receptors. *FASEB J.* 13, 1658–1676
2 Duan, D. *et al.* (2004) Conformationally constrained analogues of diacylglycerol. 21. A solid-phase method of synthesis of diacylglycerol lactones as a prelude to a combinatorial approach for the synthesis of protein kinase C isozyme-specific ligands. *J. Med. Chem.* 47, 3248–3254

Antiplasmodial activity

Malaria is the most prevalent parasitic disease in the world and affects more than 6% of the global population each year, primarily in regions between the tropics of Cancer and Capricorn [3]. Although the majority of people who contract malaria survive (after a bout of illness lasting 10–20 days), malaria kills between one and two million people annually and is one of the major causes of mortality in developing countries.

Many different drugs have been used to prevent and to treat malaria. The best-known, safest and one of the most effective antimalarials is chloroquine, a drug that is used for the treatment and chemoprophylaxis of this disease. However, because of the intensive use of

chloroquine, the parasites that cause malaria (which belong to the genus *Plasmodium*) have developed resistance to this drug. Recent research has focused on a series of novel bisbenzamidines in which the aromatic moieties are linked via a piperazine moiety to afford a conformationally restricted structure that differs from previously described analogues [3]. A small library of 11 analogues was prepared in solution as singletons. The antiplasmodial activities of the synthesized compounds were determined from the extent to which they inhibited the incorporation of [^{3}H]-hypoxanthine in nucleic acid synthesis (via the parasite purine salvage pathway). Each compound from the library was evaluated for activity against two *P. falciparum* strains (the chief



(ii)

cause of human malaria): a cloned chloroquine-susceptible strain from Haiti (Haiti 135) and a cloned chloroquine-resistant strain from Indochina (Indochina I). Results were reported as the concentrations of the test compounds required to inhibit the incorporation of [^{3}H]-hypoxanthine by 50% (IC_{50}). One of the most potent compounds identified was **ii**, which had an IC_{50} of 3 nM against the Haiti 135 strain and an IC_{50} of 4 nM against the Indochina I strain. Aromatic diamines are widely prescribed for the treatment of fungal and protozoal infections, despite their side effects. However, diamidines have not been evaluated extensively as potential antimalarials. Compound **ii** is 50-fold more active than chloroquine itself against the chloroquine-resistant Indochina I strain of *P. falciparum*. Thus, the 4,4′-(piperazine-1,4-diyl)bisbenzamidines are a promising novel class of compounds with potential antimalarial activity.

3 Mayence, A. *et al.* (2004) Parallel solution-phase synthesis of conformationally restricted congeners of pentamidine and evaluation of their antiplasmodial activities. *J. Med. Chem.* 47, 2700–2705

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Biology

Cancer Biology

Cell invasion

Several lines of evidence are growing toward the involvement of tumor–stroma interaction in cancer progression. However, pathways that lead to tumor growth, invasion and metastasis remain unfamiliar. In an article published recently in *Cancer Research* Sato *et al.* [1] used gene-expression profiling to identify the genes that are overexpressed or underexpressed during tumor growth. In a coculture of fibroblasts and pancreatic cancer cells, the tumor cells became more invasive, which is indicative of a change in gene-expression pattern.

Out of the 18,000 transcripts analyzed, <0.8 % showed either up- or downregulation in each cell type. Many genes that were pulled out via gene profiling remain to be identified, and their role in tumor–stroma interaction characterized. Nonetheless, a number of the upregulated genes identified in the current study were already known to be involved in cell migration and tumor invasion. Similarly, the downregulated genes are known to impede cancer progression.

Sato *et al.* confirmed the expression level of a subset of genes via RT–PCR. The cyclooxygenase 2 (COX-2) gene particularly attracted the attention of the authors: COX-2 is upregulated in pancreatic tumor cells and fibroblasts. It is known to be implicated in tumor metastasis and angiogenesis, and many known COX-2 inhibitors can impede cell proliferation. Thus, they addressed the effect of COX-2 inhibitor on tumor–stroma interaction and showed that the presence of specific COX-2 inhibitors in a coculture of pancreatic cancer cells and fibroblasts limits cell invasion in a concentration-dependant manner.

1 Sato, N. *et al.* (2004) Gene expression profiling of tumor-stromal interactions between pancreatic cancer cells and stromal fibroblasts. *Cancer Res.* 64, 6950–6956

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Making the transition at a Snail's pace

Epithelial-mesenchymal transition (EMT) is an important process during development that has also been implicated in metastasis. A key event is repression of E-cadherin by transcriptional regulators such as Snail. Although Snail mRNA can be present in cancer cells, the protein is often undetectable. This observation prompted researchers to investigate pathways modulating Snail protein stability [2]. Treatment of cell lines with MG132 (a proteosome inhibitor) and lithium (a GSK-3β inhibitor) resulted in synergistic elevation of Snail. This suggests that the amount of Snail protein is regulated by GSK-3β phosphorylation and proteosomal degradation.

Two GSK-3β phosphorylation motifs were identified in Snail. Site 1 overlapped a degradation site recognised by β-Trcp, whereas Site 2 appeared to enhance nuclear export. A dual-control model for regulating Snail protein stability was therefore elaborated and tested in a series of *in vitro* studies. In essence, GSK-3β binds nuclear Snail and phosphorylates Site 2, resulting in export to the cytoplasm. Subsequent modification of Site 1 by GSK-3β allows recruitment of β-Trcp and proteosomal degradation.

Numerous oncogenic signals inhibit GSK-3β. In this study, EGF abrogated the GSK-3β mediated effects on Snail. Many cancers express high levels of the EGF receptor. It is therefore possible that metastasis requires suppression of GSK-3β, resulting in stabilization and nuclear accumulation of

1359-6446/04/$ – see front matter PII: S1359-6446(04)03192-7