atherosclerosis (blood vessels grow into developing arterial plaques), diabetic retinopathy (capillaries invade the normally avascular eye), childhood hemangiomas (highly vascularized benign growths) and the growth and metastatic spread of solid tumours [Folkman, J. *Nat. Med.* (1995) 1, 27–31]. Following the pioneering work of Prof. Judah Folkman and coworkers (Harvard Medical School, Boston, MA, USA) we now know that solid tumours must constantly induce angiogenesis in order to secure the blood supply needed for continued growth. Indeed solid tumours cannot grow to above around 10^6 cells, or about the size of a pea, without inducing angiogenesis and thus establishing their own blood supply. In addition, the dissemination of cancer cells leading to secondary growths (metastasis) is closely linked to the ability of the primary tumour to induce angiogenesis, and thus its access to the vascular system, and these secondary tumours themselves can not grow successfully without inducing angiogenesis. As angiogenesis is not a continuous event in normal circumstances, molecules that can inhibit angiogenesis hold the promise of providing new noncytotoxic treatments for both primary tumours and secondary metastatic cancer, and of potentially providing new approaches to the treatment of other diseases that have an angiogenic component.

This thesis is reinforced by the work of Folkman and coworkers in the treatment of highly vascularized hemangiomas in young children with interferon-α (INFα). Long-term treatment with INFα inhibits the angiogenesis, which is a characteristic of these growths, and allows the natural regression of these lesions to occur much more quickly. The most likely mechanism for the action of INFα is based on the observation that it inhibits the production of the angiogenesis stimulatory factor, basic Fibroblast Growth Factor (bFGF). Treatment with INFα normalizes the abnormally high levels of bFGF in these children, and has saved the lives of many patients by slowing the angiogenesis-driven growth of the hemangioma and speeding natural regression.

Angiogenesis inhibitors

The endothelial cells that line blood vessels are among the slowest regenerating cells in the body, with normal cell-turnover times measured in years. During angiogenesis these endothelial cells are stimulated to proliferate much more rapidly, dividing in days and releasing a cocktail of growth factors and degradative enzymes. These stimulated endothelial cells then migrate towards the stimulus, eventually forming columns and then tubes of cells, and producing new capillary blood vessels. Based on the above rationale, medicinal chemists, biochemists and clinicians have searched for compounds that can inhibit these essential steps of angiogenesis, and several of these molecules are now in clinical trials.

TNP 470 (previously designated AGM 1470) is a semisynthetic compound derived from the natural product Fumagillin, which was discovered to be an angiogenesis inhibitor by Folkman's group. Takeda selected TNP 470 for clinical trials from a large number of synthetic analogues, based on a range of animal models indicating that TNP 470 is both less toxic and more potent than the parent Fumagillin. TNP 470 inhibits endothelial cell proliferation and migration, and shows pronounced antitumour effects in mice [Tanaka, T. *et al. Cancer Res.* (1995) 55, 836–839]. Phase I trials were initiated in late 1992, but have failed to show efficacy to date, and dose escalation is in progress. In addition, TNP 470 has now entered Phase I trials for chemotherapy-resistant relapsed childhood leukaemia, in which there is intense angiogenesis in the bone marrow, and for which there is currently no effective therapy.

Platelet factor 4 (PF4) is a natural component of platelet alpha granules, whose normal role is poorly defined. Recombinant PF4 has been shown to be angiostatic by workers at the Repligen Corp., and also to inhibit endothelial cell proliferation. Recombinant PF4 has now progressed to Phase II trials.

Thalidomide has been demonstrated recently to be orally active as an inhibitor of angiogenesis. In fact, the appalling birth defects caused by this compound may have been related to the inhibition of angiogenesis in the developing limb buds between the third and sixth weeks of pregnancy. As thalidomide is nontoxic (it is so well tolerated it does not have a measurable LD_{50}), and is orally active as an angiogenesis inhibitor, it has been entered directly into Phase II trials.

A number of other agents are currently in clinical trials as angiogenesis inhibitors, including interleukin-12 (Hoffman la Roche), Galardin (Glycomed) and BB 2576 (British Biotechnology). The effects of INFα are still being studied, and it continues to show remarkable effects in promoting the natural regression of hemangiomas in children.

Future targets

Basic research aimed at identifying new targets for angiogenesis inhibition also continues, with the integrins, which govern the interactions of endothelial cells with a variety of extracellular matrix components, currently attracting much attention. Specifically, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins seem to play key roles in the survival of newly formed vascular cells, and antagonists of the binding of these integrins are inhibitors of angiogenesis. The race is on to develop small-molecule nonpeptide antagonists of these integrin interactions.

The results of the clinical trials currently under way, and undoubtedly of others yet to be launched, will determine if the hypothesis that the inhibition of angiogenesis is a valuable new strategy in cancer therapy is valid. The very nature of the therapeutic intervention may, however, make the demonstration of efficacy for angiogenesis inhibitors very difficult under traditional protocols, and combination therapy with other more classical agents, together with new approaches, may be necessary. The drugs emerging from these trials hold the hope of a new generation of nontoxic anticancer agents, and may offer new strategies for the treatment of other diseases whose common factor is the inappropriate growth of new blood vessels.

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Combinatorial chemistry

Antifungal analogues

Synthetic solid-phase chemistry now permits the preparation of a diverse range of drug molecules. A recent paper from Bristol-Myers Squibb describes the preparation of analogues of the antifungal compound miconazole (**1**) [Tortolani, D.R. and Biller, S.A. *Tetrahedron Lett.* (1996) 37, 5687–5690]. Hydroxymethylbenzoic acid was attached to Merrifield resin and reacted with *N*-iodosuccinimide and a

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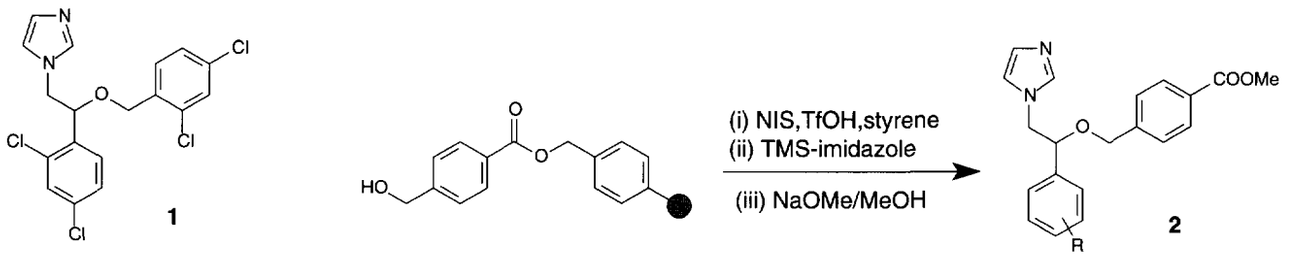

Scheme 1

range of styrenes in a key iodoetherification step. The iodoether product was derivatized with trimethylsilyl imidazole to give miconazole analogues (**2**) following cleavage with sodium methoxide (Scheme 1). A further extension of the work allowed displacement of the iodide with azide and subsequent reduction to give amines, which could then serve as a site for further diversification.

Although the yields from this route were low and variable, purities were generally high and the quantities produced were sufficient for biological assays. The route demonstrates the complexity of combinatorial synthesis currently achievable on solid-phase.

Resin capture methodologies

Armstrong has previously reported the concept of resin capture – a method by which a library synthesis commences in solution and a key intermediate is transferred to resin beads for the final step(s) [Keating, T.A. and Armstrong, R.W. *J. Am. Chem. Soc.* (1996) 118, 2574–2583]. This process effects a purification, because only suitably activated intermediates will react with the resin beads, and there is no build-up of side-products on the resin. Armstrong's group has used resin-capture for the synthesis of tetrasubstituted ethylenes related to tamoxifen (**3**) [Brown, S.D. and Armstrong, R.W. *J. Am. Chem. Soc.* (1996) 118, 6331–6332]. Bis(boryl)alkenes were monoarylated under Suzuki conditions and then captured with Rink resin-bound aryl iodide in a second Suzuki reaction, which proceeds without the addition of any further palladium catalyst (Scheme 2). The products were isolated as a mixture of regioisomers in >95% yield.

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Emerging molecular targets

RIIβ subunit of PKA and weight loss

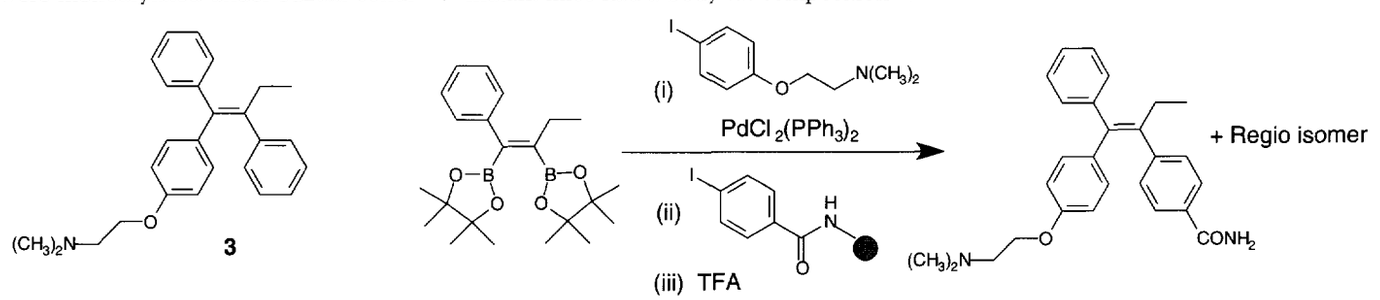
Protein kinase A is such a ubiquitous enzyme that it seems an unlikely molecular target for drug discovery of any kind, much less for anti-obesity drugs. But recent experiments from Stanley McKnight's laboratory at the University of Washington (Seattle, WA, USA) may suggest otherwise [*Nature* (1996) 382, 622–625].

Fat metabolism in adipocytes is highly regulated by protein kinase A (PKA) mediated phosphorylation. The RIIβ is the predominant regulatory subunit for PKA in brown fat, and the Seattle investigators wanted to know what would happen to fat metabolism if the RIIβ was depleted. They used targeted gene-disruption techniques to produce mutant mice that no longer produce the RIIβ subunit, and they found that the mice lost weight. The mutant mice had a body-fat composition of approximately 6% as compared to the 15% body-fat composition of the wild-type mice.

The explanation for the weight loss appears to reside in the tendency of the adipocyte to compensate for the loss of the RIIβ regulatory subunit by increasing synthesis of its isoform, the RIα subunit, which is normally present in brown fat only in very small amounts. The RIα subunit has a significantly higher affinity for cAMP than the RIIβ (K_d of 80 vs 350 nM). This results in a fivefold increase in the basal rate of activity for the mutant PKA compared with the wild-type enzyme. Overall, this causes a higher rate of basal metabolism, and much of the energy that would otherwise be stored as fat is dissipated as heat. The result is a leaner mouse.

When the wild-type mice were fed a high-fat diet over a four-month period, they became obese, while the mutant mice remained lean. But remarkably, the Seattle investigators found that the mice appeared normal in every other regard, maintaining normal plasma cholesterol, free fatty acid, insulin, glucose and thyroid hormone levels. Their results suggest that targeting the RIIβ subunit, or the transcriptional machinery that controls the ratio of the two regulatory subunits, might prove fruitful for the discovery of new anti-obesity drugs.

Robert W. Wallace



Scheme 2

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