Monitor

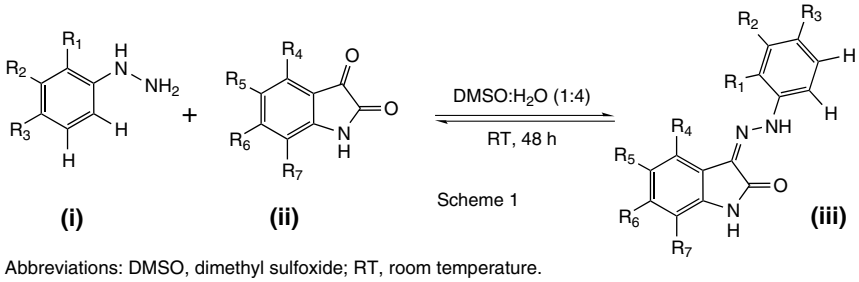
Monitor **provides an insight into the latest developments in the pharmaceutical and biotechnology industries.** ***Chemistry*** **examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while** ***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

X-ray crystallographic screening of ligands

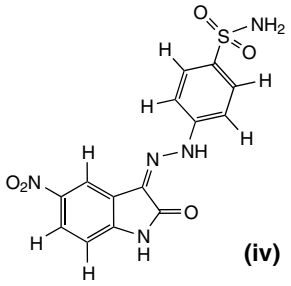
Studies have shown that the results generated by dynamic combinatorial chemistry (DCC) can be deconvoluted by X-ray crystallography, which has implications for the drug discovery process. Dynamic combinatorial chemistry (DCC) uses the binding site of a target macromolecule as a template to select and amplify specific members of a combinatorial library. In DCC, the building blocks of the template are linked together via a reversible reaction, which is in contrast to traditional combinatorial chemistry. The reversibility of the binding reaction in DCC leads to an ongoing thermodynamically controlled interchange between different members of the dynamic combinatorial library (DCL). Hence, a DCL is able to respond to molecular recognition events because the stabilisation of a particular member of the library by the template (e.g. a protein) induces a shift in the equilibrium that favours the formation of the selected species. A complimentary approach has been reported [1] that uses X-ray crystallography to characterise crystals that have been exposed to a DCL. Specifically, X-ray crystallography was used to detect potent inhibitors of the cyclin-dependent kinase 2 (CDK2) protein [1]; it has been postulated that inhibitors of CDK2 could be used for the treatment of a number of human cancers [2,3]. The technique described by Congreve *et al.* [1] facilitates the identification of the ligand of interest directly from the different members of the DCL and also enables the detailed characterisation of the binding mode of the ligand. X-ray crystallography and NMR spectroscopic techniques have been used to establish that small molecules ('fragments') of molecular weights of 100–200 are capable of binding to proteins in a reproducible manner, even though their intrinsic potency (determined by *in vitro* biological assay) is weak [4–8]. Congreve *et al.* [1] postulated that if



(i) (ii) (iii)

Scheme 1

Abbreviations: DMSO, dimethyl sulfoxide; RT, room temperature.



(iv)

fragments were bound to adjacent pockets within the active site of a protein, and they could chemically react with each other, then, potentially, the fragments could self-assemble to generate larger, more potent ligands. To explore this possibility, CDK2 was used for a proof-of-concept study [2,3]. Initial studies were performed using individual crystals of CDK2 that had been soaked in solutions containing monomers of the general structure **i** and an isatin (general structure **ii**). Under equilibrium conditions, **i** and **ii** react to produce compounds with the general structure **iii** (Scheme 1). X-ray crystallography was then used to identify Individual compounds of general structure **iii** that bound to CDK2. Next, these compounds were resynthesised, purified and their inhibitory concentrations measured by an assay typically used for the determination of CDK2 activity. One of the most potent compounds identified was **iv**, which has an IC_{50} of 30 nM. These studies demonstrate that it is possible to use X-ray crystallography to detect small-molecule ligands that are generated *in situ* and then

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bind to a target protein. Hence, this approach provides a basis for a new drug discovery technology that uses DCL to identify novel, potent ligands. This technology could be applicable to the discovery of inhibitors of other therapeutically useful proteins.

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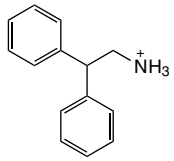
Binders to the angiogenesis marker fibronectin

The formation of new blood vessels from preexisting blood vessels (angiogenesis) is an essential prerequisite for the growth of the majority of aggressive solid tumours but is a rare event in adult physiology, with the exception of the female reproductive cycle. Overexuberant angiogenesis is a characteristic feature of blinding occular disorders (e.g. diabetic retinopathy and age-related macular degeneration) and rheumatoid arthritis. Therefore, markers of angiogenesis represent an ideal target for molecular intervention in, for example, cancer. Fibronectin is a multidomain adhesive glycoprotein that is abundant in plasma and tissues. Alternative splicing of the primary transcript of fibronectin results in the production of several different isoforms of this glycoprotein. One of the extra domains (ED) of fibronectin is ED-B, which comprises a 91 amino acid sequence that is identical in mouse, rat and human fibronectin proteins. ED-B-containing fibronectin (B-FN) has a restricted pattern of expression and is undetectable in normal adult tissues and in mature blood vessels. However, B-FN accumulates in the regenerating tissue around new blood vessels. As a result of the high level of conservation of the ED-B sequence, the generation of monoclonal antibodies by immunisation has been unsuccessful to date [9]. In addition to high-throughput methods of drug discovery, some groups are using screening methodologies that are based on 2D-heteronuclear NMR spectroscopy for protein targets of molecular weights less than 30,000 Da [9]. A marked advantage of using target-based NMR screening methodologies over conventional HTS is the information that is gained about the binding site for the drug under investigation. However, NMR screening methods have low throughput and require substantial amounts of both protein and ligand. Nevertheless, the structural information acquired in the process could be invaluable for, for example, the design of focused affinity-matured libraries of lead compounds. A recent study [10] used NMR screening of a small, rationally designed library of low molecular-weight compounds to identify lead compounds that bind specifically to the ED-B domain of fibronectin. Eighty-five compounds were tested in groups of five compounds for binding to ^{15}N-labelled ED-B. The spectrum of each mixture was recorded and compared with a reference spectrum of ^{15}N-labelled ED-B alone. From this comparison, it was observed that one particular mixture produced a large shift in the resonance of the signal from a single backbone amide group. Deconvolution of this mixture, followed by the re-screening of the five compounds individually, led to the identification of the small molecule **v** as the most potent binder to ED-B, which had a dissociation constant K_d in excess of 5 mM. This work has provided a small molecule starting point for the design of multidentate ED-B binders with improved affinity and this approach warrants further investigation.



(v)

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Paul Edwards
paul.edwards@graffinity.com

Biology

Cancer biology

A novel mechanism of anti-estrogen resistance

Anti-estrogens inhibit estrogen receptor-positive breast cancers by arresting cell growth. However, treatment is associated with resistance. In order to improve therapies, it is therefore essential to understand the processes underlying acquired drug resistance.

ACTR is a nuclear hormone receptor co-activator that is overexpressed in breast cancer. While investigating the role of this protein in proliferation, workers in the laboratory of Hong-Wu Chen uncovered a novel mechanism of anti-estrogen resistance [1].

In the presence or absence of estradiol, depletion of ACTR with siRNA inhibited proliferation, while increased expression enhanced cell growth. Remarkably, elevation of ACTR also promoted proliferation in the presence of anti-estrogens. Proliferation correlated with increased cells in S phase and a selective induction of E2F1 target genes critical for the G1/S transition.

Chromatin immunoprecipitation demonstrated that ACTR could bind the promoters of E2F1-regulated genes, suggesting that it may be an E2F1 co-activator. Accordingly, ACTR augmented E2F1 mediated transactivation in promoter-reporter assays. E2F1 bound directly to ACTR via an N-terminal region

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