ELSEVIER

monitor

MONITOR

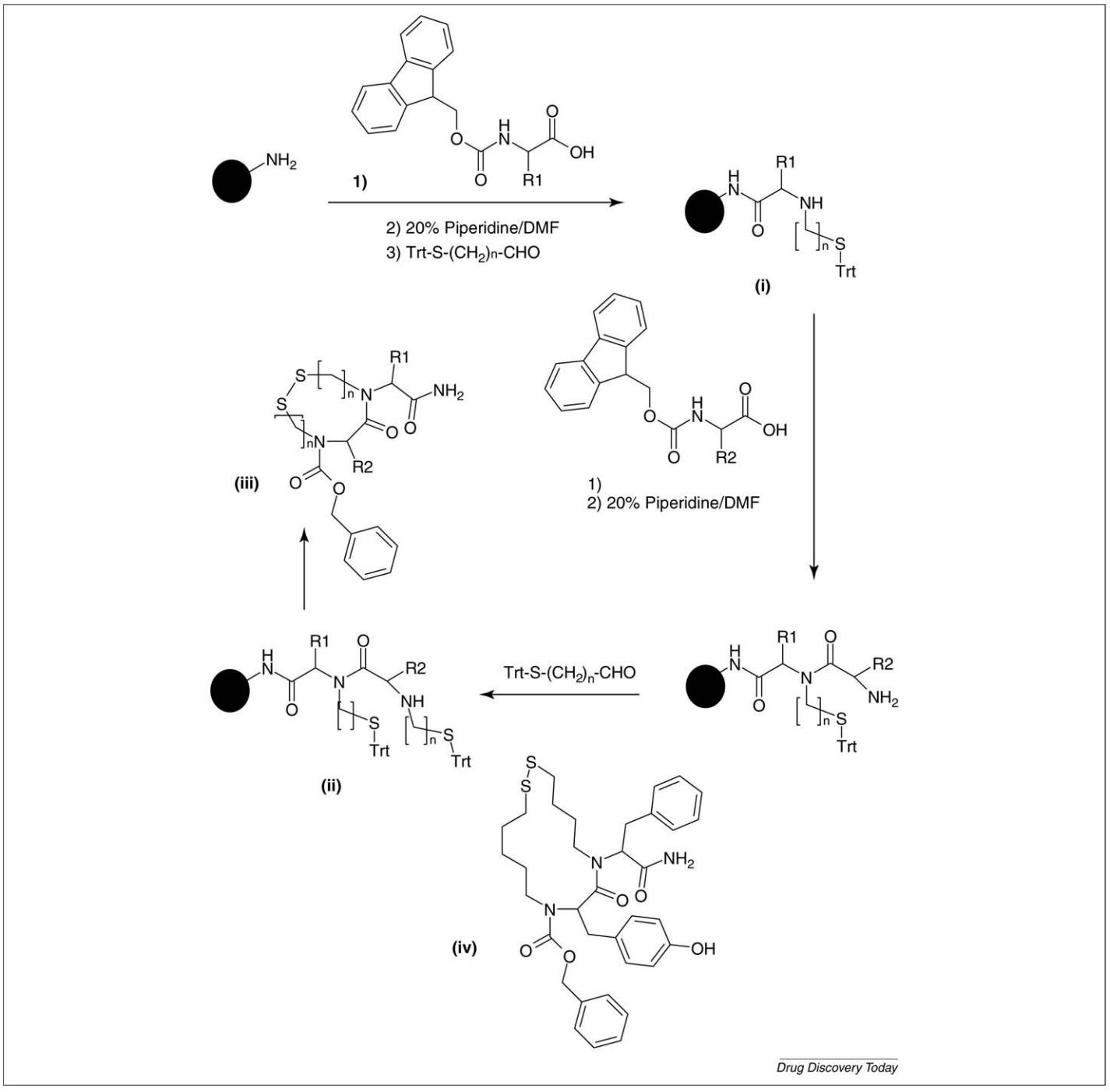
The design and synthesis of libraries for the discovery of biologically active substances

Synthesis of a macrocyclic library and discovery of IGF-1R inhibitors

Insulin-like growth factor-1 (IGF-1) receptor (IGF-1R) [1] is often overexpressed in a broad spectrum of malignancies, such as cancers of the prostate, pancreas, colon and lung [2]. The activity of IGF-1R is enhanced in cancers owing to upregulation of its ligand IGF-1. Insulin-like growth factor-1 receptor activates several signaling pathways, including the extracellular signal-regulated kinase (ERK) pathway, as well as others. It signals for proliferation and protection against apoptotic stimuli, as well as resistance to cytotoxic drugs [3]. Insulin-like growth factor-1 receptor contributes to cell transformation and to early-stage tumor development, as well as during the metastatic phase. Many researchers have, therefore, recognized insulin-like growth factor-1 receptor as an attractive target for cancer therapy and attempted to produce inhibitors of its activity or expression. It is possible to arrive at compounds with suitable biological activity from screening natural products or chemical libraries. In this regard, naturally occurring compounds can be used as templates for creating designed combinatorial libraries based on active sequences of peptides and proteins [4]. A limitation with such approaches, using these types of scaffold, can be the intrinsic limitation of conformational space sampled, thus preventing the pharmacophores from reaching their bioactive conformation. The use of linear peptides as drugs is often problematic because of their conformational flexibility, susceptibility to enzymatic degradation, and low bioavailability. Cyclization can be used to overcome these limitations because it restricts the conformational freedom of peptides and improves their metabolic stability and selectivity toward receptors [5]. The use of cyclic peptides as a scaffold for the pharmacophoric moiety, however, often fails because the constrained scaffold prevents the pharmacophores from achieving a bioactive conformation. This failure may be overcome by synthesizing combinatorial libraries of small cyclic molecules in which the scaffold (pharmacophores) are varied and thus presented in various conformations.

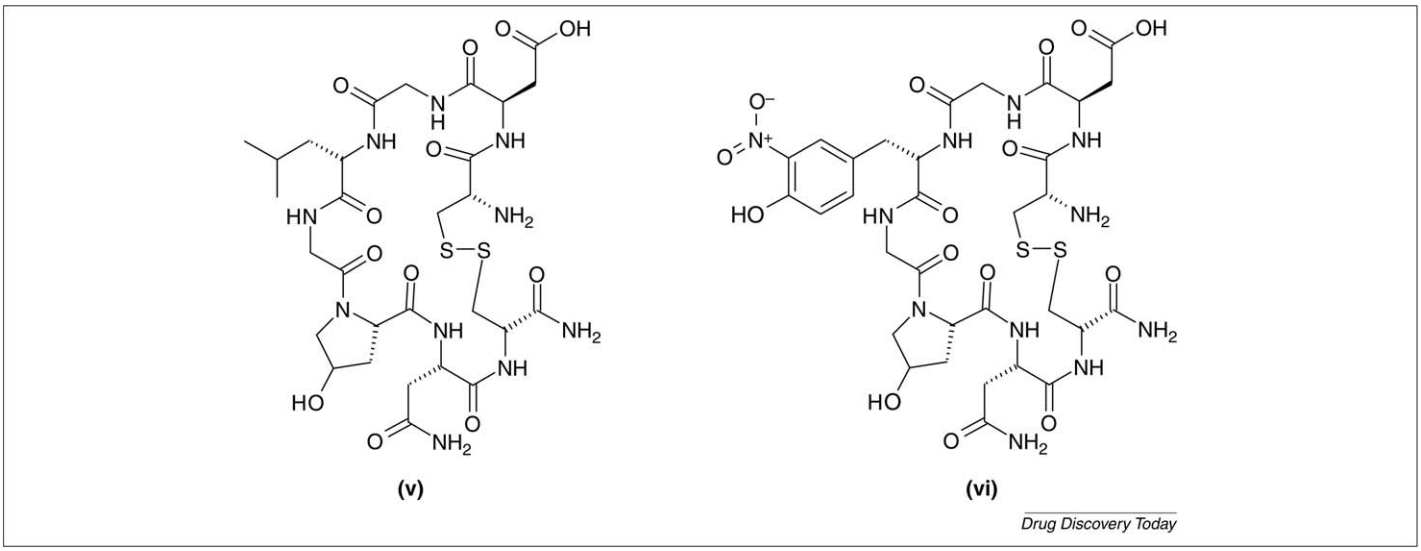
Recent work has disclosed the synthesis of a combinatorial library of small macrocyclic molecules based on pharmacophores derived from the activation loop of insulin-like growth factor-1 receptor, in order to inhibit the activation and the tyrosine kinase activity of insulin-like growth factor-1 receptor [6]. Two library protocols were designed for creation of compounds for biological screening. At this stage of protocol design, synthesis used the Merrifield vessel method [7] and the Houghten tea bag method [8]. For the execution of the library itself, the tea bag method combinatorial approach was selected because it was easier and faster to synthesize a diverse library using this method. The library was synthesized by loading of an amino acid onto the solid phase, removal of the Fmoc-protecting group using piperidine, followed by a reductive amination coupling of an aldehyde with the liberated resin-bound primary amine, to deliver compounds of general structure (i). This methodology was repeated a second time to deliver compounds of general structure (ii). These were then subjected to an acylation of the secondary amine in (ii), cyclization and cleavage from resin to deliver compounds of general structure (iii). In this way a 34-membered library was synthesized. Liberated compounds were then screened in crude form for their inhibitory action in two independent assays. Firstly, for inhibition of IGF1-induced IGF-1R phosphorylation in breast cancer MCF7 cells at 20 μM concentration, and for inhibition of anchorage-dependent growth of MCF7 cells at various concentrations of each compound. A number of active compounds were obtained from screening this library. One of the most potent was (iv) which possessed an IC_{50} of 6 μM for inhibition of the IGF-1-induced IGF-1R autophosphorylation in MCF-7 breast cancer cells. Although in this work the synthesis of the library compounds suffered from low yields, the benefit of this approach is that the resulting library has a single set of pharmacophores of a known active site with varying scaffold sizes. This allows systematic screening of the bioactive conformations of a given set of pharmacophores in the search of drug leads, ultimately leading to (iv) in the present work. This concept could be useful in cases where the pharmacophores are known but need to be systematically screened for a spatial arrangement that will enable biological activity. Further work in this area is warranted to both broaden the SAR and understanding of physicochemical properties in this series, and to generate an improved synthetic methodology that may enable a wider use of the concept in the future.

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2010.08.011



Targeting ligands for breast cancer cells using the One-Bead One-Compound combinatorial method

Breast cancer is the most common malignant tumor among women, accounting for an estimated 24% of all cancer cases [9]. Despite the current treatments (chemotherapy, radiation therapy, surgery), breast cancer remains the second most lethal cancer for women (18% of all cancer deaths). A major reason for its aggressive nature is its tendency to metastasize even before the disease can be detected clinically or through screening [10]. Estrogen receptor (ER)/progesterone receptor (PR) negative breast cancers tend to be very aggressive, and both taxane-based chemotherapy and radiation therapy remain the main medical treatment options. Radioimmunotherapy using a ^{131}I-labeled monoclonal antibody against tumor cells has shown promise in early clinical studies, but this agent possesses limitations of non-specific uptake of the antibody molecules by the reticuloendothelial system in sites such as the liver and spleen, leading to dose-limiting toxicities [11]. An approach to obviate the problem of radioimmunoconjugates or immunotoxins is to develop peptide-based cancer cell surface or tumor neovascular cell surface targeting agents to deliver radionuclides, toxins, or cytotoxic agents to the tumor site. The peptides used are usually derived from binding motifs of known proteins or from phage-displayed peptide libraries, both of which are limited to L-amino acids and, thus, are susceptible to proteolysis. It is possible to minimize the problems of proteolysis by using a 'one-bead one-compound' (OBOC) encoded combinatorial library method to design and prepare libraries with, for example, peptides blocked at the N-terminal, cyclic peptides, peptidomimetics, small molecules, and macrocyclic molecules. [12]. In OBOC libraries, each resin bead displays a unique peptide, and



millions of library beads can be screened in parallel against the biological target of interest [12]. The positive beads are then physically isolated for structural determination by microsequencing using automatic Edman degradation. Recent work [13] has described the use of OBOC combinational chemistry and high-throughput on-bead cell binding assays to identify novel breast cancer targeting ligands. In this work, several random linear and cyclic OBOC peptide libraries were synthesized and screened against an ER-negative breast cancer cell line (MDA-MB-231). A cyclic peptide with the sequence cEGLGEWc was shown to bind to the MDA-MB-231 breast cancer cells. Based on this result, an OBOC cyclic peptide with a cX1GX3GX5X6c motif was designed and synthesized. In this library, the X1 position was diversified with 42 natural and unnatural amino acids including D-amino acids. Each position of X3, X5, and X6 contained 1 of 41 natural and unnatural L-amino acids. This library was screened against MDA-MB-231 breast cancer cells using a standard whole-cell binding assay. About 3×10^5 beads (400 μL of settled beads) were screened, and 19 positive beads were isolated for microsequencing. Three further OBOC libraries were prepared and screened against MDA-MB-231 breast cancer cells. Arising from this work was the discovery of a novel cyclic peptide (v). This compound was shown to possess a binding affinity (K_d) to the R3 integrin on MDA-MB-231 breast cancer cells of approximately 0.4 μM. Molecular interactions between the R3 integrin and (v) were characterized by using a series of K562 cells transfected with various mutant R3 integrins. Using established structure–activity relationships, two highly focused cyclic peptide libraries were then designed, synthesized, and screened against MDA-MB-231 breast cancer cells. A novel cyclic peptide (vi) with an improved binding affinity was identified. This compound possessed an IC_{50} of 57 nM. This work is of importance because through the screening of four OBOC peptide libraries against live MDA-MB-231 cells, a series of peptide ligands were identified. Additionally, important structure and activity relationships were elucidated. This work has resulted in the discovery of a peptide ligand (vi). Further work in this area is warranted to evaluate the binding profile of compounds derived from these libraries against cancer cells obtained directly from primary breast cancer tumors. The ultimate aim will be the use of compounds such as (vi) in the efficient vehicle delivery of radionuclides, cytotoxic agents, or drug-loaded nanoparticles for breast cancer imaging and therapy.

1 Blum, G. *et al.* (2003) Development of new insulin-like growth factor-1 receptor kinase inhibitors using catechol mimics. *J. Biol. Chem.* 278, 40442–40454
2 Bohula, E.A. *et al.* (2003) Targeting the type 1 insulin-like growth factor receptor as anti-cancer treatment. *Anticancer Drugs* 14, 669–682
3 Resnicoff, M. (1995) The insulin-like growth factor I receptor protects tumor cells from apoptosis in vivo. *Cancer Res.* 55, 2463–2469
4 Hall, D.G. *et al.* (2001) Solution- and solid-phase strategies for the design, synthesis, and screening of libraries based on natural product templates: a comprehensive survey. *J. Comb. Chem.* 3, 125–150
5 Ludesche, U. and Schwyzer, R. (1971) Chirality of the cystine disulfide group. Assignment of helical sense in a model compound with a dihedral angle greater than ninety degrees using NMR and CD. *Helv. Chim. Acta* 54, 1637–1644
6 Qvit, N. *et al.* (2008) Synthesis of a novel macrocyclic library: discovery of an IGF-1R inhibitor. *J. Comb. Chem.* 10, 256–266
7 Merrifield, R.B. (1963) Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* 85, 2149–2154
8 Houghten, R.A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. U.S.A.* 82, 5131–5135
9 Van de Wiele, C. *et al.* (2000) Radiolabeled estradiol derivatives to predict response to hormonal treatment in breast cancer: a review. *Eur. J. Nucl. Med.* 27, 1421–1433
10 Epidemiology of breast cancer. In The breast: *comprehensive management of benign and malignant diseases* (Vincent, G.F., Bland, K.I., Copeland, E.M., eds), 1998. pp. 339–351, Saunders, Philadelphia
11 Neumeister, P. *et al.* (2001) Hepatic veno-occlusive disease in two patients with relapsed acute myeloid leukemia treated with anti CD33 calicheamicin (CMA-676) immunoconjugate. *Ann. Hematol.* 80, 119–120
12 Lam, K.S. *et al.* (1997) The "one-bead-one-compound" combinatorial library method. *Chem. Rev.* 97, 411–448
13 Yao, N. *et al.* (2009) Discovery of targeting ligands for breast cancer cells using the One-Bead One-Compound combinatorial method. *J. Med. Chem.* 52, 126–133

Paul Edwards
mepauledwards@fsmail.net