A recent review has discussed the potential use of cathepsin K inhibitors as an alternative to oestrogen replacement therapy for the treatment of postmenopausal osteoporosis [Smith, W.W. and Abdel-Meguid, S.S. (1999) *Exp. Opin. Ther. Patents* 9, 683–694]. The review highlights the successful application of modern drug discovery processes through reference to recent papers and patents, including genomics and structure-based drug design, the identification of this novel molecular target, and rapid identification of novel, potent and selective inhibitors of cathepsin K.

Inhibitors of this cysteine protease might also have uses in adjuvant therapy following the implantation of orthopaedic implants where inhibition of bone resorption might offer a means of enhancing the rate of osteointegration of implanted devices.

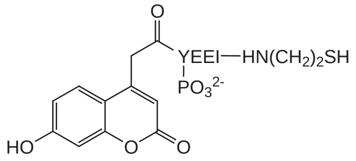
Andrew Lloyd

Combinatorial chemistry

High-affinity SH2-targeted ligands

The Src-homology-2 (SH2) domain plays a key role in the recognition of specific protein sequences that contain a phosphotyrosine residue. Attempts to design agents that bind to the SH2 domain has been driven by a desire to interrupt signalling pathways that are known to be responsible for various diseases. Although the affinity of peptides for the SH2 domain lies in the 200–800 nM range, non-peptidic ligands are generally one to three orders of magnitude less active.

A recent publication describes the parallel solid-phase synthesis of peptide and non-peptide conjugates that have a high affinity for the SH2 domains of LCK and FYN [Lee, T.R. and Lawrence, D.S. (1999) *J. Med. Chem.* 42, 784–787]. Using the Fmoc-protection protocol, a phosphotyrosine-containing peptide attached to the solid phase through a disulfide linker was derivatized by a range of carboxylic acids to generate 900 analogues. Cleavage of the products allowed screening of their ability to bind to SH2 domains using an enzyme-linked immunosorbent assay (ELISA). Although the majority of compounds failed to display any affinity in the ELISA assay, several compounds, including the coumarin derivative (**1**) and the sulfonated compound (**2**), are among the most potent SH2-targeted agents yet identified.



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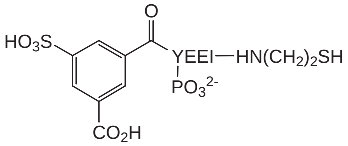

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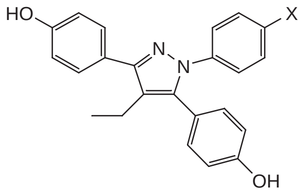
These two key compounds also demonstrated an unprecedented ability to discriminate between the SH2 domains of two members of the Src family of protein kinases.

Oestrogen-receptor ligands

Natural and synthetic compounds affecting oestrogen levels have an important role as agents for the control of fertility, hormone-responsive breast cancer and for menopausal hormone replacement therapy. Most synthetic oestrogens retain a phenolic function, but there are many other synthetic oestrogens including both cyclic and acyclic systems. A key goal of medicinal chemistry has been to obtain agents that have high levels of tissue selectivity. The latest chapter in this process is the design of novel oestrogen-receptor ligand-templates that can be synthesized by simple condensation reactions typical of those used in combinatorial chemistry [Fink, B.E. *et al.* (1999) *Chem. Biol.* 6, 205–219].

Many oestrogen-receptor ligands display a central core structure containing peripheral structural elements and in this paper, a number of 1,2- and 1,3-azole heterocycles (imidazoles, thiazoles, oxazoles and pyrazoles) were prepared and screened as receptor ligands. The binding values, obtained from a competitive radiometric-binding assay using tritiated oestradiol, demonstrated that of all these systems, pyrazoles had the highest binding affinity. In particular, compounds (**3**) and (**4**) had high levels of binding and showed close conformational relationships to the non-steroidal ligand, raloxifene. The solid-phase synthesis of related pyrazole libraries is under way in the search for more potent and selective oestrogen-receptor ligands.

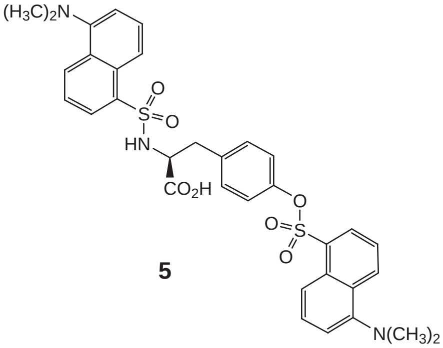


3 X = H
4 X = OH

Thymidylate synthase inhibitors

Thymidylate synthase (TS) catalyzes the final step in the biochemical pathway leading to thymidylate and has been nominated as a target for the discovery of new anticancer and antimicrobial drugs. The X-ray structure of TS obtained from *Lactobacillus casei* (LcTS) has been derived, and was the starting point for the design of novel library-derived inhibitors [Tondi, D. *et al.* (1999) *Chem. Biol.* 6, 319–331]. 153,516 compounds from the available chemicals directory were screened against the X-ray structure using the molecular-docking program, DOCK, and five high-ranking compounds were selected for screening. Of these, dansyltyrosine was selected for solid-phase synthesis of 33 analogues.

One analogue discovered, didansyltyrosine (**5**), has a K_i value of 1.3 μM, demonstrating that combinatorial chemistry and parallel synthesis techniques are complementary to structure-based methods for the discovery of novel enzyme inhibitors that have no obvious similarity to the enzyme substrate.



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Potential use of infrared microspectroscopy to study drug-related structural changes in bone

Bone tissue is a complex structure resulting from the intense activities of many cell lineages (e.g. osteoblasts, osteoclasts). The different cell types are interconnected and communicate via soluble or membranous mediators. Thus, a very large cytokine network provides for bone development and allows bone integrity to be conserved during life. Such mediators (cytokines, hormones and ions) can be released from the bone matrix as a result of osteoclastic bone resorption, a component of the normal remodelling process necessary to maintain bone integrity.

Calcium phosphate (Ca-P)-mineral phases of bone are composed of a poorly carbonated apatite. These phases play a key role in the mechanical properties of the bone as well as in several biological processes (such as homeostasis of phosphocalcic metabolism and cell regulation), and can be altered by Ca-P mineral metabolism disorders. Bisphosphonates, analogues of endogenous pyrophosphates, are highly potent inhibitors of osteoclastic bone resorption and decrease tumour cell adhesion and represent an important class of drugs for the treatment of patients with bone diseases. However, bisphosphonates are known to be avidly bound to hydroxyapatite crystals and can alter the chemical composition and structure of biological apatite crystals of bone. In addition, the long biological half-life of bisphosphonates might be detrimental to the metabolism of the skeleton.

Although bone is one of the most common metastatic sites in human breast and prostate cancers, the molecular mechanisms by which tumour cells induce osteolytic metastases are still not fully understood. It is accepted that tumour cell adhesion to the bone matrix and the release of soluble mediators from tumour cells stimulate bone resorption. Newly synthesized drugs, hormones and some cytokines that could be used for therapeutic purposes in bone disease might affect bone crystal structure and consequently its mechanical and biological functions.

Infrared spectroscopy has provided important information on the fine structure and the physicochemical characteristics of biological apatite-like bone. Information can be obtained concerning the nature of the mineral phases and their mineral content (i.e. phosphate, carbonate), mineral crystallinity and maturity, and the content of the organic matrix (secondary structure of proteins).

The application of resolution-enhancement techniques and curve-fitting methods has led to qualitative and semi-quantitative information regarding PO_4^{3-}, HPO_4^{2-} and CO_3^{2-} molecular environments. This information has been found to provide very sensitive indicators of bone mineral maturation and alterations in bone metabolism [Rey, C. *et al.* (1995) *Cells Mater.* 5, 345–356] and in the organic matrix structure [Paschalis, E.P. *et al.* (1997) *J. Bone Miner. Res.* 12(Suppl.), 229]. Similar information is also available at the microscopic level using infrared microspectroscopy. This information provides valuable molecular information on the chemistry of tissues and their spatial variations (i.e. mapping) at defined anatomical and morphological locations from tissue sections [Bohic, S. *et al.* (1998) *C. R. Acad. Sci. Paris Life Sci.* 321, 865–876; Boskey, A.L. *et al.* (1998) *Bone* 23, 187–196; Kalasinsky, V.F. *et al.* (1996) *App. Spectrosc. Rev.* 31, 193–249; LeVine, S.M. *et al.* (1994) *Am. J. Pathol.* 145, 1041–1047].

The combination of Fourier-transform infrared microspectroscopy (FT-IR) spectroscopy and light microscopy enables infrared transmission analysis of sections (0.5–5.0 μm thick) of embedded biological samples placed on suitable non-absorbing infrared windows, or examination, by reflectance, of the bulk sample placed on a gold mirror. The sample microstructure can be viewed from histology sections through the microscope and sample areas are selected prior to infrared analysis. Chemical mapping can be generated with 10 μm spatial resolution using a motorized stage moving the sample to sequential sites while the infrared beam remains stationary. Infrared spectra can also be recorded from discrete sites of the sections. The methodology for infrared microspectroscopic analysis can be obtained from the papers described later. The utility of infrared microspectroscopy relative to other methodologies for investigating calcified tissues, is that these tissues do not need to be homogenized. In fact, with infrared spectroscopy and X-ray diffraction,

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