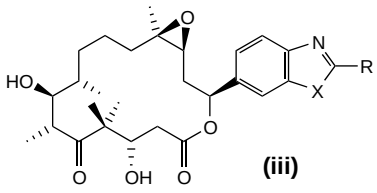
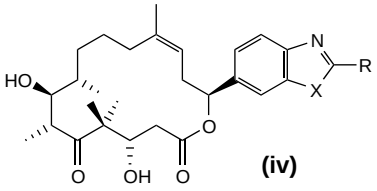
Highly potent analogues of epothilones B and D

In recent years, the natural product epothilones A, B, C and D have emerged as a new class of microtubule-stabilizing agents with potent *in vitro* and *in vivo* antitumour activity, resulting in a host of research activity directed at structure–activity relationship studies and the design and synthesis of novel, potent epothilone analogues. Altmann and coworkers at Novartis Pharma AG (Basel, Switzerland) report the synthesis of a new class of epothilone B and D analogues in which the natural (2-(2-methylthiazol-4-yl)-1-methyl)-ethenyl side-chain has been replaced with several benzoheterocyclic moieties **(iii)** and **(iv)**[3]. This new class of agents show more potent anti-proliferative activity than epothilones B and D in the human epidermoid cancer cell-line KB-31 and a P-glycoprotein-overexpressing, paclitaxel-resistant subline KB-8511.



(iii)



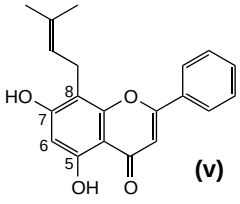
(iv)

R = CH_3; X = S
R = H; X = N(CH_3)
R = CH_3; X = N(CH_3)
R = H; X = CH = CH

3 Altmann, K-H. *et al.* (2000) Synthesis and biological evaluation of highly potent analogues of epothilones B and D. *Bioorg. Med. Chem. Lett.* 10, 2765–2768

Enhancement of binding affinity toward P-glycoprotein and modulation of cancer cell chemoresistance

Multidrug resistance (MDR) of cancer cells is often correlated with the overexpression of P-glycoprotein (P-gp), a membrane transporter protein that rejects several cytotoxic drugs (e.g. anthracyclines, vinca alkaloids, taxanes and epipodophyllotoxins) from cells. Flavonoids have been reported to act as P-gp modulators by mimicking the adenine moiety of ATP, the energy source for P-gp. Barron and coworkers in Lyon (France) have reported the synthesis of a series of *C*- or *O*-substituted hydrophobic derivatives of chrysin (5,7-dihydroxyflavone) to investigate the structural requirements and the effect of increasing the hydrophobicity of the A-ring toward P-gp modulation[4]. Increasing the hydrophobicity at either position 6, 7 or 8 increased the affinity of *in vitro* binding to a purified cytosolic domain of P-gp, but only benzyl and 3,3-dimethylallyl *C*-substitution produced a high maximal quenching of the protein intrinsic-fluorescence. Inhibition of membrane P-gp within leukaemic cells using 8-(3,3-dimethylallyl)chrysin **(v)** was found to be more efficient than for the commonly used cyclosporin A.



(v)

4 Barron, D. *et al.* (2001) *C*-Isoprenylation of flavonoids enhances binding affinity toward P-glycoprotein and modulation of cancer cell chemoresistance. *J. Med. Chem.* 44, 763–768

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Drug delivery

Bone-specific delivery of diclofenac

Prodrug strategies have considerable potential as site-specific drug delivery systems. There are many site-specific drug delivery strategies, but osseous tissues are still difficult to target because of the biological properties of bone. Osseous tissues consist mainly of the inorganic compound hydroxyapatite (HAP), and bones lack the efficient circulatory systems of other tissues, with blood-flow rates in bone of 0.05–0.2 ml min^{-1} g^{-1}. Bisphosphonates are a class of synthetic compounds that are structurally related to pyrophosphate and are clinically used to treat various bone disorders, including osteoporosis. Bisphosphonates are known to have high affinity for HAP, and osseous tissues are the main targets for the accumulation of bisphosphonates in the body after administration. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely employed as painkillers. However, NSAIDs have several undesirable side effects, in particular gastrointestinal (GI) toxicity. The NSAID diclofenac (DIC) is used clinically in the treatment of rheumatism, but to obtain therapeutic effects, large doses of DIC, which are known to cause GI damage, are required. The bone-specific delivery of NSAIDs, therefore, could be beneficial in the treatment of bone disease, while decreasing undesirable GI side effects.

Hirabayashi and colleagues have recently proposed a drug-delivery system that targets osseous tissues based on a bisphosphonic prodrug moiety[1]. This strategy was previously demonstrated using carboxyfluorescein as a model drug. The bisphosphonic prodrug of carboxyfluorescein (CF–BP) maintains the osteotropic property of the bisphosphonic moiety. CF–BP is rapidly taken up into osseous tissues after intravenous injection and disappears slowly, with a half-life of ~26 days. The successful use of this bisphosphonic prodrug strategy has now been reported for bone-specific delivery and sustained release of DIC. The bisphosphonic prodrug of DIC, disodium 2-(2,6-dichloroanilino)phenylacetoxyacetoaminomethylene bisphosphonate (DIC-BP) was synthesized by linking DIC to an aminomethylene

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bisphosphonate via an acetic-acid linker. The biodistribution characteristics and elimination rate from bone of DIC–BP were then examined by *in-vivo* studies with Sprague-Dawley rats. The skeletal distribution of DIC–BP was extrapolated from the amount detected in the femur, in proportion to the femur's contribution to total skeleton weight.

Rats received DIC–BP intravenously at doses of 0.32, 1.0, 3.2 and 10.0 mg kg^{-1}. At an appropriate time after administration, blood samples were collected. After the last sampling, the rats were sacrificed, and the brain, lungs, heart, kidneys, liver, spleen and femur were excised and assayed to determine drug concentration. At all doses, the amount of DIC–BP remaining in plasma was <2% of the dose when tissue samples were collected. DIC–BP was detected in the femur, liver and spleen, but the concentrations of DIC–BP in the brain, heart, lungs and kidneys were under the limit of detection. DIC–BP was taken up into the skeleton at all doses but tended to increase as a fraction of dose with increasing dose. The amounts of DIC–BP recovered in the skeleton were: 57.9 ± 13.0, 54.1 ± 3.3, 38.3 ± 6.4 and 24.8 ± 1.4% of administered dose at 0.32, 1.0, 3.2 and 10.0 mg kg^{-1}, respectively. The liver was another target for the accumulation of DIC–BP; the amounts recovered in the liver were 21.8 ± 1.1, 18.3 ± 0.7, 23.5 ± 2.3 and 38.9 ± 1.2% of the administered dose at doses of 0.32, 1.0, 3.2 and 10 mg kg^{-1}, respectively. Thus, the liver was the major target-organ for the disposition of DIC–BP at a dose of 10 mg kg^{-1}. The investigators believe that this effect is closely related to the propensity of bisphosphonate drugs to precipitate with metal ions (i.e. iron and calcium), and that this can be controlled by maintaining a low dose to avoid the precipitation effects.

For the determination of elimination rate from bone, a separate group of rats received a 10 mg kg^{-1} intravenous dose of DIC–BP. After an appropriate time (up to 28 days) post-injection, blood samples were collected, the animal was sacrificed, and the femur was excised. DIC–BP was detected in the femur over the entire 28 days of the experiment. The peak localization of DIC–BP was observed within 8 h after dosing. After 8 h, the skeletal concentration of DIC–BP declined in a biphasic manner with half-lives of 3.8 days in the early phase (from 8 h to 2 days) and 9.7 days in the terminal phase (from 4 to 28 days). Sustained release of regenerated DIC into the bone compartment was observed over the entire experimental period, and the bone concentration of regenerated DIC was constant throughout the 28 days.

Finally, the therapeutic and side effects of DIC–BP were compared with those of DIC in an adjuvant-induced arthritic rat model. DIC-sodium salt was administered daily by the oral route and DIC-BP was administered weekly by the intravenous route. For both DIC and DIC–BP, inhibitory effects against the swelling of rat paws were observed after a dose of 0.32 mg kg^{-1}, and arthritic scores detected in rat paws and tail were improved after a dose of 3.2 mg kg^{-1}. Both effects increased in a dose-dependent manner. Taking into account the frequency of medication (17 doses for DIC–sodium and four doses for DIC–BP in the experimental period), ED_{50} values of DIC and DIC-BP were corrected to 9.4 and 5.2 mg kg^{-1} (per experimental period), respectively. DIC treatment caused GI damage, even at the lowest dose of 0.032 mg kg^{-1}, with mean ulceration doses of 0.8 and 3.7 mg kg^{-1} for stomach and intestine, respectively. DIC–BP showed an improved side-effect profile: neither stomach nor intestinal ulcers were observed in rats treated with DIC–BP. These early studies did not address an oral formulation of DIC–BP, but it is significant that the sustained release of DIC into bone from DIC–BP permitted effective once weekly intravenous dosing. The bone-specific delivery and sustained release properties of DIC-BP could enhance the pharmacological effects of DIC for bone disease, while simultaneously preventing adverse GI effects and increasing patient compliance by a decrease in the frequency of administration.

1 Hirabayashi, H. *et al.* (2001) Bone-specific delivery and sustained release of diclofenac, a non-steroidal anti-inflammatory drug, via bisphosphonic prodrug based on the Osteotropic Drug Delivery System (ODDS). *J. Control. Release* 70, 183–191

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Erratum

Please note a correction to 'Current progress on new therapies for Alzheimer's disease' by Patrick C. May published in *Drug Discovery Today* 6(9), 459–462. On page 461, first column, the last two lines should have read 'Oral administration of one of the BMS inhibitors, dose undefined...'

The Editorial team of *Drug Discovery Today* would like to apologize for this inaccuracy and for any confusion that we might have caused.