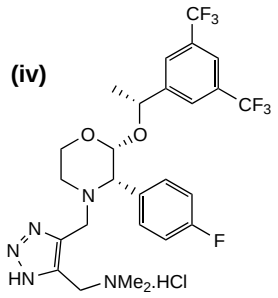
ml^{-1}) and >3000-fold selectivity against h-NK_2, h-NK_3 and 100 other receptors, ion channels and enzymes.

(iv)



In a guinea pig hind-foot-tapping model the compound displayed excellent CNS penetration and duration of action (ID_{50} = 0.3 mg kg^{-1} for 24 h intravenous pretreatment) for blockade of a centrally acting NK_1 receptor agonist. Compound **(iv)** was shown to inhibit retching and vomiting in the ferret induced by cytotoxic agents such as cisplatin and centrally acting agents such as morphine. The effect was observed both for intravenous dosing (ID_{90} = 0.1 mg kg^{-1}) and oral dosing as an aqueous solution (ID_{90} = 1 mg kg^{-1}).

Anti-depressant activity was observed in a guinea pig neonatal vocalization model (ID_{50} = 0.2 mg kg^{-1} perorally). The compound is thus highly effective in preclinical tests for emesis and depression.

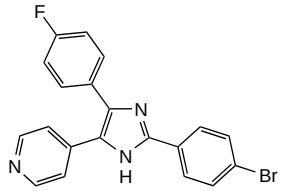
3 Hale, J.J. *et al.* (1998) Structural optimization affording 2-(*R*)-1(*R*)-3,5-*bis*(trifluoromethyl)phenylethoxy)-3-(*S*)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine, a potent orally active long-acting morpholine acetal human NK_1 receptor antagonist. *J. Med. Chem.* 41, 4607–4614

4 Harrison, T. *et al.* (2001) An orally active, water soluble neurokinin-1 receptor antagonist suitable for both intravenous and oral clinical administration. *J. Med. Chem.* 44, 4296–4299

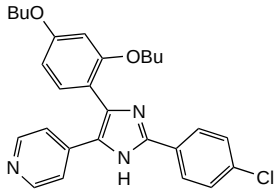
Imidazoles as glucagon receptor antagonists

The morbidity associated with non-insulin dependent diabetes (NIDDM) is in large part a result of prolonged elevated levels of plasma glucose. Glucagon is a peptide hormone that counters the effects of insulin to maintain glucose homeostasis. The hormone causes an increase in hepatic gluconeogenesis, glycogenolysis and reduces the ability of insulin to inhibit these processes. Antagonists of the glucagon receptor are thus anticipated to decrease the rate of glucose release from the liver and improve the response to insulin.

A group from the Merck research laboratories (Rahway, NJ, USA) undertook a screening effort to identify non-peptide antagonists of the human glucagon receptor (h-Glur) [5]. The triaryl imidazole **(v)** was identified (IC_{50} = 0.27 μM) but it is also an inhibitor of p38 mitogen-activated protein kinase (MAPK; IC_{50} = 0.16 μM).



(v)



(vi)

Investigation into the structure–activity relationships (SARs) showed the requirement for both the imidazole NH and the 4-pyridyl nitrogen atom for binding to the glucagon receptor. It was found that substitution of the 4-aryl group improved binding to the receptor and also conferred selectivity for the glucagon receptor over MAPK. Compound **(vi)** was identified (IC_{50} = 0.006 μM, h-Glur) and exhibited only 20% inhibition at 40 μM for p38 MAPK. In the presence of a physiological concentration of Mg^{2+} ions (5 mM) the activity of the compounds in the series dropped 2–25 fold for binding to h-Glur. In the presence of a physiological concentration of Mg^{2+} the activity of **(vi)** was 0.053 μM against h-Glur.

Thus by systematic SAR studies a selective small molecule was identified to help investigate the role of h-Glur antagonism in glucose homeostasis.

5 Chang, L.L. *et al.* (2001) Substuted imidazoles as glucagon receptor antagonists. *Bioorg. Med. Chem. Lett.* 11, 2549–2553

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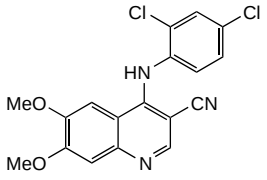
Novel antitumour molecules

Potent inhibitors of src kinase activity

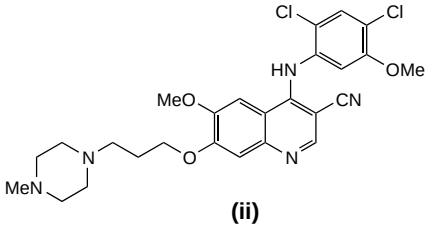
Tyrosine kinases (TKs) are enzymes that catalyze the specific phosphorylation of tyrosine residues on proteins. The highly homologous Src TK family are involved in several intracellular signalling pathways and are overexpressed in several human tumours, notably metastatic tumours. Src TKs are, therefore, a valid target for anticancer drug development. In addition, Src has been implicated in vascular endothelial growth factor (VEGF) signalling in endothelial cells, implying a possible anti-angiogenesis role for Src kinase. Src inhibitors might also have a role in the prevention of brain damage following stroke and in the treatment of osteoporosis.

Boschelli and coworkers at Wyeth-Ayerst Research (New York, NY, USA) have described the optimization of 4-phenylamino-3-quinolinecarbonitriles as potent inhibitors of Src activity [1]. Using the previously described Src kinase inhibitor 4-[(2,4-dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile [compound **(i)**; IC_{50} = 30 nM] as a starting point, several rounds of refinement of this lead compound through SAR studies led to the identification of compound

(ii), which had an IC_{50} value of 1.2 nM in the Src enzymatic assay, an IC_{50} value of 100 nM for the inhibition of Src-dependent cell proliferation and was selective for Src over non-Src family kinases. Evaluation of compound **(ii)** in xenograft models employing Src-transformed fibroblasts indicated that in a staged model, where tumours were implanted three days before dosing, a 25 mg kg^{-1} dose administered twice a day for ten days, provided a T:C of 25%, indicating that compound **(ii)** is sufficiently bioavailable to inhibit Src *in vivo*.



(i)



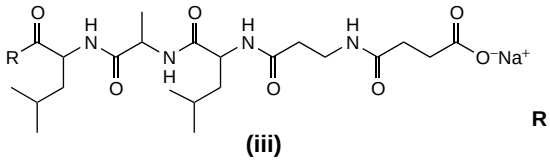
(ii)

1 Boschelli, D.H. *et al.* (2001) Optimization of 4-phenylamino-3-quinolinecarbonitriles as potent inhibitors of Src kinase activity. *J. Med. Chem.* 44, 3965–3977

Doxorubicin prodrugs

Doxorubicin has a broad spectrum of antitumour activity and has a significant role in the treatment of solid tumours, such as carcinoma of the breast, lung, thyroid and ovary. Substantial research efforts are being made to overcome dose-limiting side effects of, and resistance to, drugs such as doxorubicin, through the more specific delivery of cytotoxic agents to tumour cells, at the same time sparing effects on normal cells. Two recent publications focusing on doxorubicin serve to illustrate this approach.

Fernandez and coworkers at the Université Catholique de Louvain (Louvain, Belgium) and Corixa Corporation (San Francisco, CA, USA) have described the application of a new extracellularly tumour-activated doxorubicin prodrug devoid of intravenous acute toxicity [2]. This approach was based on the previously described prodrug *N*-(β-alanyl-L-leucyl-L-alanyl-L-leucyl)doxorubicin that is stable in blood and unable to enter cells until activated by peptidases released by tumour cells. N-β-(alanyl-L-leucyl-L-alanyl-L-leucyl)doxorubicin displays reduced *in vivo* toxicity and increased efficacy, compared with doxorubicin alone when administered in two human breast tumour xenograft models. However, intravenous administration of the prodrug in this model induced an acute toxic reaction, resulting from its positive charge at physiological pH combined with its propensity to form large aggregates in aqueous solutions. The same authors have now discovered that a negatively charged *N*-capped succinyl derivative **(iii)**, can be administered by the intravenous route at more than ten times the LD_{50} of doxorubicin without inducing the acute toxic reaction; the new derivative **(iii)** is also active *in vivo* (MCF-7 human breast tumour xenografts in mice).



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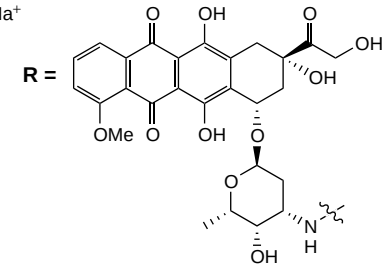

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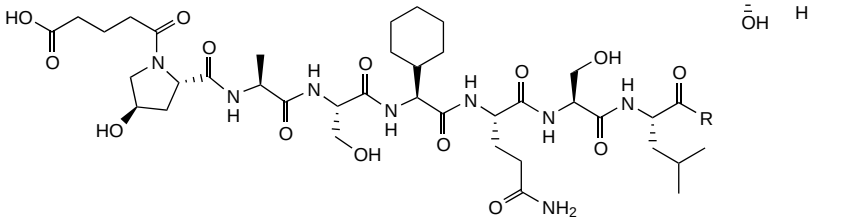


(iv)

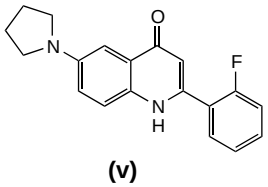
In a second recent publication concerning doxorubicin prodrugs, Garsky and coworkers described the synthesis of a doxorubicin-peptide conjugate specifically designed to be hydrolyzed by the serine protease, prostate specific antigen (PSA) [3]. Serum PSA levels have been found to correlate well with the number of malignant prostate cells; in addition, PSA, which is secreted into systemic circulation, lacks enzymatic activity. The use of a prodrug cleaved by PSA in the prostate should therefore provide high localized concentrations of doxorubicin at the tumour site, and at the same time sparing systemic exposure to the cytotoxic drug. The doxorubicin-peptide conjugates were evaluated as targeted prodrugs for PSA-secreting tumour cells, from which Glutaryl-Hyp-Ala-Ser-Chg-Gln-Ser-Leu-Doxorubicin, compound **(iv)** emerged as the most promising candidate. Compound **(iv)** was found to have higher than 20-fold selectivity against human prostate PSA-secreting LNCaP cells relative to the non-PSA-secreting DuPRO cell line. Also, in nude mouse xenograft studies using human LNCaP prostate cancer cells, **(iv)** reduced PSA levels by 95% and tumour weight by 87% at a dose below its maximum tolerated dose.

2 Fernandez, A-M. *et al.* (2001) *N*-Succinyl-(β-alanyl-L-leucyl-L-alanyl-L-leucyl)doxorubicin: an extracellularly tumor-activated prodrug devoid of intravenous acute toxicity. *J. Med. Chem.* 44, 3750–3753

3 Garsky, V.M. *et al.* (2001) The synthesis of a prodrug of doxorubicin designed to provide reduced systemic toxicity and greater target efficacy. *J. Med. Chem.* 44, 4216–4224

Fluorinated 2-phenyl-4-quinolone derivatives as antimitotic antitumour agents

The formation of microtubules and the mitotic spindle is a crucial event in cellular replication and microtubules have, in recent years, become an important subcellular target for the development of novel anticancer agents. Well-known agents in this respect include the *Vinca* alkloids, taxoids and colchicines. Xia and coworkers, at the University of North Carolina and the National Cancer Institute (Frederick and Bethesda, MD, USA) have extended their studies on the 2-phenyl-4-quinolones as new antimitotic antitumour agents and now report the synthesis and antitumour evaluation of a series of fluorinated 2-phenyl-4-quinolones [4]. The compounds were tested in the National Cancer Institute (NCI) 60 human tumour cell line *in vitro* screen and a good correlation was found between cytotoxicity and inhibition of tubulin polymerization. Among the new compounds tested, 2′-fluoro-6-pyrrol-2-phenyl-4-quinolone, compound **(v)**, exhibited the most potent cytotoxic activities (log GI_{50} <–8.00) against renal and melanoma tumour cell lines. Compound **(v)** was also found to be a potent inhibitor of tubulin polymerisation (IC_{50} = 0.46 μM) with activities comparable to the potent antimitotic natural products colchicines, podophyllotoxin and combretastatin A-4.



(v)

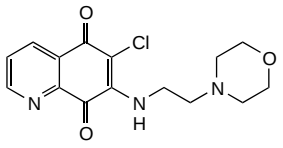
4 Xia, Y. *et al.* (2001) Antitumor agents. 211. Fluorinated 2-phenyl-4-quinolone derivatives as antimitotic antitumor agents. *J. Med. Chem.* 44, 3932–3936

Potent inhibitors of the dual specificity protein phosphatase Cdc25

Reversible protein phosphorylation (of serine, threonine or tyrosine) is a ubiquitous intracellular process in cellular communication and growth, determined by the dynamic balance between protein kinases and phosphatases. Natural product inhibitors of the serine and threonine phosphatases are known, however, there is an absence of potent and selective inhibitors of protein tyrosine phosphatases (PTPases), which include the dual specificity protein phosphatase (DSPase) subfamily. Important members of the DSPase subfamily, particularly with regard to the design of anticancer agents, are the Cdc25 phosphatases, which control cell cycle progression by activating cyclin-dependent kinases (Cdks) and participate in Raf-1-mediated cell signalling.

Of the three Cdc25 homologues that exist in humans (Cdc25A, Cdc25B and Cdc25C), Cdc25A and B have oncogenic properties, are transcriptional targets of the *c-myc* oncogene and are overexpressed in many human tumours. The Cdc25 homologues also possess a number of other cellular functions including regulation of cell cycle progression through activation of Cdk and cyclin complexes, DNA damage response, and Raf-1 and mitogen-activated kinase regulation. Taken together, these observations provide some validation of Cdc25s as anticancer drug targets.

Lazo and coworkers from the University of Pittsburgh (Pittsburgh, PA, USA), have experimentally examined the 1990 compound National Cancer Institute (NCI) Diversity Set (designed to be representative of the 140,000 NCI compound repository) for Cdc25 inhibitors from which eight quinolinediones emerged that had *in vitro* IC_{50} values of <1 μM [5]. The most potent compound was found to be 6-chloro-7-(2-morpholin-4-ylethylamino)quinoline-5,8-dione, compound **(vi)**, which was 20- and 450-fold more selective for $Cdc25B_2$, compared with the related phosphatases VHR or PTP1B respectively. Compound **(vi)** also blocked cell proliferation *in vitro* in human breast cancer MDA-MB-435 and MDA-N cells (IC_{50} = 0.2 μM) and, using a chemical complementation assay, was found to block cellular Erk dephosphorylation caused by ectopic Cdc25A expression.



(vi)

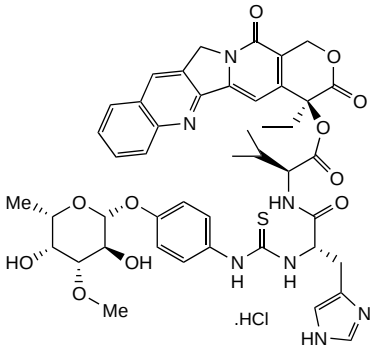
5 Lazo, J.S. *et al.* (2001) Discovery and biological evaluation of a new family of potent inhibitors of the dual specificity protein phosphatase Cdc25. *J. Med. Chem.* 44, 4042–4049

Camptothecin glycoconjugates

Camptothecin is a pentacyclic alkaloid that has demonstrated impressive activity against leukaemias and a variety of solid tumours. Although camptothecin itself has been withdrawn from clinical studies due to dose-limiting toxicities, several analogues have either been approved for clinical use (irinotecan and topotecan) or are currently in clinical trials, and the camptothecin field continues to attract interest. Several targeted prodrug and delivery systems for camptothecins have been developed, including 20-O-alkyl esters, simple amino acid salts and polymeric delivery systems [polyethylene glycol (PEG) and *N*-2-hydroxypropylmethacylamide (HPMA) conjugates].

A major issue of the camptothecin class of compounds is the opening of the lactone E-ring and the formation of an equilibrium between the ring closed lactone form (active) and open carboxylate form (inactive). To improve the biological profile of camptothecin, Lerchen and coworkers at Bayer AG (Wuppertal, Germany) have synthesized a novel class of 20-*O*-linked camptothecin glycoconjugates for preferential cellular uptake into tumour cells by an active transport mechanism [6]. The conjugates have been optimized for enhanced water solubility, stabilization of the active camptothecin lactone ring form, and sufficient

hydrolytic and proteolytic stability. The constitution of the peptide spacer between camptothecin and the carbohydrate end was found to have a major influence on stability and biological activity of the conjugates; glycoconjugates with valine residues at the linkage position to camptothecin were found to be sufficiently stable and active *in vitro* against colon HT29 and other human tumour cell lines. In particular, glycoconjugate **(vii)** was found to be optimal amongst those examined, inhibiting tumour growth by >96% in the breast cancer xenograft model and comparing



(vii)

favourably to topotecan with respect to toxicity against hematopoietic stem cells and hepatocytes. Glycoconjugate **(vii)** has been selected for clinical trials on the basis of the above considerations.

6 Lerchen, H-G. *et al.* (2001) Design and optimisation of 20-*O*-linked camptothecin glycoconjugates as anticancer agents. *J. Med. Chem.* 44, 4186–4195

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