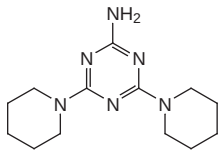
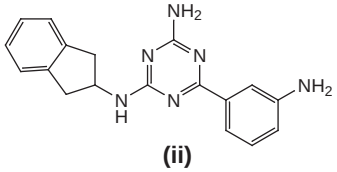
methylation are the erythromycin-resistance methylase (Erm)-family of methyltransferases, and inhibition of these enzymes sensitizes MLS-resistant bacteria to macrolide antibiotics.



(i)



(ii)

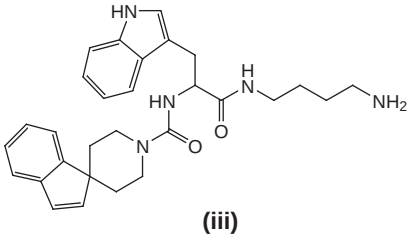
In the search for novel and selective inhibitors of ErmAM methyltransferase using structure–activity relationship studies (SAR) by NMR, several small organic molecules have been found[6]. In particular, compound (**i**) was discovered to have a K_i of 75 μM. Solution-phase parallel synthesis was then employed to independently optimize the piperidine substituents, resulting in compounds, such as (**ii**), that have low micromolar affinity for the ErmAM methyltransferase. Because of their non-nucleoside structure, they are potentially selective inhibitors of Erm methyltransferase that could be given in combination with a broad-spectrum macrolide antibiotic.

6 Hajduk, P.J. *et al.* (1999) Novel inhibitors of Erm methyltransferases from NMR and parallel synthesis. *J. Med. Chem.* 42, 3852–3859

Somatostatin-receptor ligands

Somatostatin, a ubiquitous tetradecapeptide, is involved in a range of different biological functions including modulation of the secretion of growth hormone, insulin, glucagon and gastric acid. Five different human somatostatin receptors have been cloned and characterized, offering the potential for the design of novel and selective non-peptide ligands.

To find such ligands, database searching techniques have been used to screen the Merck-compound sample collection using the modelled conformation of a known potent cyclic peptide mimetic of somatostatin[7]. Of the 75 compounds selected, L264930 (**iii**) was selected as a high-affinity ligand suitable for combinatorial optimization.



(iii)

The compound can readily be considered to comprise three parts, and a mix-and-split solid-phase synthetic protocol was used to prepare an expected 131,670 compounds in 79 mixtures of 1330 or 2660 products. A semi-automated procedure was used for the screening of the library in a 96-well plate format. Following deconvolution, several potent ligands selective for the somatostatin receptor subtypes (K_i = 50 pM to 200 nM) were discovered.

7 Berk, S.C. *et al.* (1999) A combinatorial approach toward the discovery of non-peptide, subtype-selective somatostatin receptor ligands. *J. Comb. Chem.* 1, 388–396

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High-throughput screening

Rapid fluorescent-based reporter-gene assays for HTS of platinum-based cytotoxic agents

Although cisplatin and carboplatin are widely used in chemotherapy, many tumours are resistant to these compounds. This has led to an interest in rapidly screening combinatorial libraries of cisplatin analogues to assess their potential use as antitumour agents. Two high-throughput fluorescent-based reporter-gene assays have recently been described that might offer a means of screening cisplatin analogues and related compounds for antitumor activity[8].

One reporter-gene assay used HeLa Tet-On cells that were transfected with the doxycycline-inducible enhanced green fluorescent protein (GFP) gene. A highly inducible, low background clone was isolated and used to evaluate the effects of cisplatin, cisplatin analogues and other cytotoxic stress enhancers on GFP expression. Cisplatin and other *cis*-disubstituted platinum complexes inhibited GFP expression whilst alternative forms of cytotoxic stress stimulated GFP transcription. The other reporter-gene assay used the hydrolysis of the fluorescent cephalosporin substrate CCF2 to monitor β-lactamase expression in Jurkat cells. Using this assay, cisplatin was found to inhibit the β-lactamase expression whereas $[Pt(NH_3)_2Cl_2]$ and $K_2(PtCl_4)$ were not.

As these assays provide results more rapidly than conventional cytotoxicity assays, they might offer a means, in conjunction with combinatorial chemistry, of accelerating the discovery of novel anticancer agents.

8 Sandman, K. *et al.* (1999) Rapid fluorescence-based reporter-gene assays to evaluate the cytotoxicity and antitumor drug potential of platinum complexes. *Chem. Biol.* 6, 541–551

Andrew Lloyd

New safer non-steroidal anti-inflammatory drugs?

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely

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prescribed medicines worldwide and represent a milestone therapy for many inflammatory diseases, as well as being used extensively in over-the-counter preparations[1]. However, their major disadvantage is the high incidence of gastric adverse effects, caused by the blockade of prostaglandin (PG) synthesis in the stomach. To circumvent this problem, which also represents a financial burden on the health care system, several strategies have been developed, including the administration of NSAIDs in enteric coated formulations or as prodrugs, and concomitant therapy with antisecretory or gastroprotective drugs. These drugs, however, either do not offer complete protection against NSAID-induced gastric damage or produce adverse effects in a significant number of patients.

In recent years, selective inhibitors of the inducible isoform of cyclooxygenase (COX-2) have been developed that should inhibit inflammatory PGs produced by the COX-2 enzyme, while sparing constitutive (COX-1 produced) PGs in the stomach[2]. Experimental and clinical results indicate that these drugs offer some advantages over the available NSAIDs, which either do not discriminate between the two COX isoforms or are actually selective COX-1 inhibitors. Recent data, however, suggest that COX-2 might also be necessary for the protection of the stomach while COX-1 might also be involved in the inflammation process, thus indicating that more knowledge is necessary before the definite roles of the COX isoforms in the different tissues can be assessed[3].

An alternative approach has been the use of nitric oxide (NO)-associated NSAIDs. The underlying rational was based on the observations that NO has been recently recognized as a fundamental mediator of gastric mucosal defense, sharing many gastroprotective effects with PGs, namely stimulation of mucus secretion, maintenance of mucosal blood flow, and inhibition of leukocyte adhesion to the vascular endothelium. Thus, NSAIDs that release NO could have the advantage of sparing the stomach, by opposing the negative effects caused by PG suppression. So far, experimental and clinical studies have indeed demonstrated that NO–NSAIDs are much better tolerated than NSAIDs alone[4]. A further recent approach, also based on the protective effects of NO, is offered by NSAIDs that increase NO synthesis in the gastric mucosa.

Amtolmetin guacyl

Workers from Sigma-Tau (Pomezia, Italy) and Medosan (Pomezia, Italy) have recently developed a novel NSAID, namely amtolmetin guacyl, which is characterized by a greatly reduced gastric toxicity both in rodents and in humans[5]. Histological studies in rats have demonstrated that this drug, unlike classical NSAIDs, did not induce haemorrhagic or necrotic lesions, even when administered intragastrically at doses up to sixfold higher than those necessary to reduce cutaneous inflammation[6]. An electron microscopy study in rats showed that gastric microcirculation is well preserved, with no signs of vasocongestion. Moreover, there was no evidence of leukocyte adhesion to the vascular endothelium. This finding is very important when considering that neutrophil adherence, with consequent vascular occlusion and free radical release, is considered to be the crucial event in the pathogenesis of NSAID-induced mucosal injury[7]. Amtolmetin guacyl did not alter gastric transmucosal potential difference in the anaesthetized rat, suggesting that mucosal barrier integrity was maintained. By contrast, classical NSAIDs, such as aspirin, ibuprofen and indomethacin, markedly reduced gastric potential difference, suggesting disruption of the mucosal barrier. Studies directed to clarify the mechanism of the reduced toxicity of amtolmetin guacyl indicated that this new NSAID caused a sustained activation of NO synthase in the gastric mucosa[8]. It is reasonable to hypothesize that the beneficial role exerted by NO in the gastric mucosa might counterbalance the negative effects caused by PG suppression. In some experimental models, amtolmetin guacyl actually reduced gastric damage induced by ethanol and this protective effect was abolished by NO synthase inhibitors. Clinical data already available confirm the experimental findings and suggest that this drug will provide a useful alternative to existing NSAIDs in the treatment of inflammatory disorders.

Unlike NO donor-NSAIDs, amtolmetin guacyl increased NO production predominantly in the stomach. In fact, this compound is rapidly hydrolized by plasmatic estherases into tolmetin and tolmetin glycinamide, two metabolites that are unable to increase gastric NO production. The selective increase of NO in the stomach and not in other tissues might have useful clinical implications, when considering that NO can exert a broad range of biological effects that can be detrimental in some tissues.

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Validation of genomics-derived drug targets using yeast

The steady flow of novel genes from genomics provides a great opportunity for increasing the number of therapeutic targets, a limited resource for those engaged in drug discovery. The paucity of valid drug targets limits the impact the pharmaceutical and biotechnological industries can have on disease states and places constraints on the future improvement of health care worldwide. Realization of the potential of a decoded human genome will require a concerted effort by researchers to elucidate the physiological roles of newly identified proteins in normal and diseased processes. As the investigation of fundamental disease mechanisms is time-intensive, strategies that accelerate the evaluation of candidate molecules as disease-relevant targets must be devised.

The broad impact of genomics on drug development will be expeditiously handled only by establishing a clear focus, whether that is within a molecular class, a therapeutic area, an organ system, a type of tissue or a disease process. In addition, it will be important to take advantage of any strategy that is able to side-step obstacles to target validation. The yeast-based system described here is an example of a focused, alternative approach designed to expedite the process of moving from the DNA sequence to its biological relevance to the production of drugs.

Saccharomyces cerevisiae

The yeast *Saccharomyces cerevisiae* is being exploited to obtain the tools required for discerning whether a given orphan G protein-coupled receptor (GPCR) is a suitable drug target. The prediction that the growing collection of orphan GPCRs will be a rich source of targets for therapeutic intervention is based on the recognition that a significant portion of the drugs currently on the market target this receptor family[1]. The potential functional areas of GPCRs for which natural ligands have recently been identified also support the prediction (Table 1). For example, with the identification of the motilin receptor[2] comes the possibility of new treatments for gastrointestinal disorders.

Surrogate ligands

To embark on an investigation of receptor biology, it might be ideal to have the natural ligand, but this is not essential. Surrogate ligands have facilitated receptor research in the past (e.g. nicotine, muscarine, NMDA, kainate) and are especially useful in enabling target validation in this era of genomics, where ligand identification is lagging, at times by many years, behind the cloning of new receptors. A potent, selective surrogate agonist enables the elucidation of signalling pathways, an assessment of biological function and the discovery of receptor antagonists. These tools, agonist and antagonist, facilitate the study of receptor function in the absence of the natural ligand.

Functional expression of mammalian homologues

Yeasts are amenable to the functional expression of mammalian homologues of yeast proteins. This fact, together with the rich history of yeast genetics and the completion of the sequencing of the yeast genome, has made *Saccharomyces cerevisiae* an ideal organism to use in augmenting other approaches to target validation. Yeast screens can be engineered that permit rapid identification of surrogate agonists and receptor antagonists of orphan GPCRs while offering distinct advantages. By deleting endogenous pheromone receptors, yeasts can be made to contain a null background for the expression of human receptors. This

Table 1. 'De-orphanized' G protein-coupled receptors

Receptor	Ligand	Predominant receptor localization	Potential functional area
GPR38/MTL-R1A	Motilin	Duodenum, colon	Gastrointestinal motility
GPR9–GPR6	TECK	Thymus	Thymocyte development
Edg-1	Sphingosine-1-phosphate	Endothelial cells	Angiogenesis
HGFAN72/OX$_1$R	Orexin	Brain	Feeding
HGR3	Prolactin-releasing peptide	Pituitary	Pregnancy, lactation
APJ	Apelin	CNS	Unknown

Abbreviations: GPR, G-protein receptor; HGR, human glucocorticoid receptor; MTLB, mannitol regulator; TECK, thymus-expressed chemokine.

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