Monitor: molecules and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

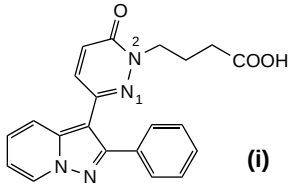
Molecules

Orally active adenosine A_1 receptor antagonists that permeate the blood–brain barrier

In the CNS, adenosine regulates a variety of important physiological functions via the G-protein-coupled extracellular adenosine receptors, which are classified into four subtypes A_1, A_{2A}, A_{2B} and A_3. All of the four human subtypes have been cloned[1]. The A_1 and A_{2A} receptors are high affinity receptors for adenosine, whereas the A_{2B} and A_3 are low-affinity receptors.

Researchers from Fujisawa Pharmaceutical Co. (Osaka, Japan) has recently reported the discovery of compound FK838 as a potent A_1 receptor antagonist provided with strong diuretic activity[2]. Although initially the inventors hoped the compound might be useful for CNS-related diseases, FK838 **(i)** was not detected in the brain after peroral (p.o.) administration, despite the fact that it has good oral bioavailability. This was thought to be a result of poor blood–brain barrier penetration. The same group has now reported on a novel series of 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines, structurally related to FK838 (Ref. 3).

In particular, because the poor brain-permeability of FK838 was attributed to the presence of the carboxylic group in its side chain, the new derivatives were synthesized to have different functional groups, for example, carbonyl, hydroxy, amide, amine and piperidine at the N2 position. All of the compounds were evaluated for their *in vitro* A_1 and A_{2A} human receptor-binding affinity. Selectivity was expressed as the ratio of K_i values (A_{2A}:A_1). Stability to *in vitro* metabolism in rat liver microsomes and cytosol was also measured.

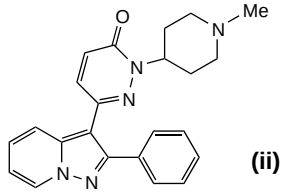


(i)

The most interesting compounds were examined for plasma and brain concentrations after p.o. administration to rats and the brain:plasma ratio (B:P) evaluated. The structure–activity relationship (SAR) study of ~30 compounds showed that:

(1) A carbonyl group is a good substituent for high A_1 affinity (its replacement by a hydroxy or amide decreased both affinity and selectivity);
(2) Mono-substituted amides showed a higher A_1 affinity and selectivity than di-substituted and cyclic amides;
(3) The piperidine derivatives, introduction of a lipophilic moiety reduced A_1 affinity but retained A_{2A} affinity; and
(4) Lipophilicity is an important factor for A_1 affinity, with a suitable clogP value being in the range 1.5–3.5.

Compound **(ii)** (A_{2A}:A_1 = 820), which showed a good brain concentration and good B:P ratio at a dose of 3.2 mg kg^{-1} p.o. after 30 min from administration (concentration in plasma and brain = 389 and 361 nM, respectively; B:P = 0.93), was selected as a good candidate for further pharmacological evaluations.

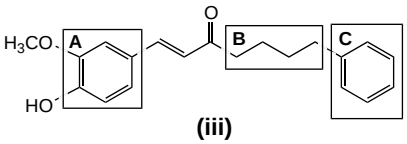


(ii)

1 Fredholm, B.B. *et al.* (1994) Nomenclature and classification of purinoceptors. *Pharmacol. Rev.* 46, 143–156
2 Akahane, A. *et al.* (1999) Discovery of 6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinebutanoic acid (FK838): a novel non-xanthine adenosine A1 receptor antagonist with potent diuretic activity. *J. Med. Chem.* 42, 779–783
3 Kuroda, S. *et al.* (2001) Design, synthesis and biological evaluation of a novel series of potent, orally active adenosine A_1 receptor antagonists with high blood–brain barrier permeability. *Chem. Pharm. Bull.* 49, 988–998

Novel acyl-CoA:cholesterol-*O*-acyltransferase (ACAT) inhibitors

Since the Framingham study[4] hypercholesterolemia has been well-recognized as a major risk factor for the development of coronary heart disease (CHD). Therefore, agents controlling total plasma-cholesterol levels are expected to



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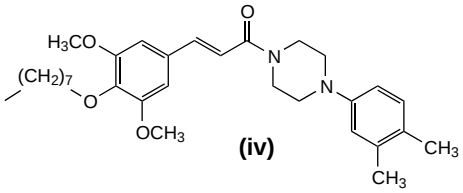


(iv)

be effective in the treatment of atherosclerosis. Acyl-CoA:cholesterol-*O*-acyltransferase (ACAT, EC 2.3.1.26) has important roles in the absorption of dietary cholesterol from the small intestine, the secretion of very-low-density lipoprotein (VLDL) from the liver, and the accumulation of cholesteryl esters in atherosclerotic lesions. Therefore, ACAT inhibitors have been investigated as therapeutic agents for hypercholesterolemia and atherosclerosis.

By screening metabolites from ~3000 fungi and many plant components, the 1-(4-hydroxy-3-methoxyphenyl)-7-phenylhept-1-en-3-one (**iii**; Yakuchinone B), a component of the seeds of *Alpinia oxyphylla*, was found to have ACAT inhibitory activity (IC_{50} = 20.6 μM in rat hepatic microsomes)[5].

Ohishi and collaborators have recently published their results on a series of novel ACAT inhibitors[6], which were derived from **iii**, by introducing progressive modifications on the three structural moieties A, B and C. The compounds were tested for their ability to inhibit microsomal ACAT from rat liver and to suppress the elevation of plasma-cholesterol levels in rats given a high cholesterol diet. Of almost 50 derivatives prepared, many had IC_{50} values in the nanomolar range. The *N*-(3,5-dimethoxy-4-*n*-octyloxycinnamoyl)-*N′*-(3,4-dimethylphenyl) piperazine (**iv**) (IC_{50} = 11 nM) inhibited rat hepatic ACAT better than the established inhibitors CI976 and YM750 (IC_{50} = 98 and 55 nM, respectively)[6] and had higher hypocholesterolemic activity (ED_{50} values = 2.4, 5.1 and 4.2 mg kg d^{-1} for **iv**, CI976 and YM750, respectively).

It should also be noted that **iv** strongly inhibited the microsomal ACAT prepared from human cell lines (Caco 2 IC_{50} = 63 nM and HepG2 IC_{50} = 88 nM), which suggests that the compound might be able to inhibit ACAT in both the human gastrointestinal tract and the liver, regardless of the pattern of expression of the ACAT isozymes.

4 Kannel, W.B. *et al.* (1971) Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. *Ann. Intern. Med.* 74, 1–12

5 Itokawa, H. *et al.* (1982) A pungent principle from *Alpinia oxyphylla*. *Phytochemistry* 21, 241–243

6 Ohishi, K. *et al.* (2001) Structure–activity relationships of *N*-(3,5-dimethoxy-4-*n*-octyloxycinnamoyl)-*N′*-(3,4-dimethylphenyl)piperazine and analogues as inhibitors of acyl-CoA:cholesterol-*O*-acyltransferase. *Chem. Pharm. Bull.* 49, 830–839

Non-peptide small molecules that inhibit binding of insulin-like growth factor (IGF) to IGF-binding proteins

The insulin-like growth factors (IGF-I and II) are small polypeptide hormones regulating cell proliferation, cell differentiation, cell death and cell metabolic activities[7]. Their action is mediated by their binding to and activation of the cell surface IGF-I receptor, an $\alpha_2\beta_2$ heterotetramer that is closely related to the insulin receptor. Upon ligand binding, the IGF-I receptor changes its conformation, resulting in autophosphorylation of the intracellular β-subunits and activation of the receptor's intrinsic tyrosine-kinase activity to propagate the signal to the nucleus[8]. In blood and interstitial fluids the concentration of freely circulating IGF is exceedingly low because most of the IGFs are bound to one or more of six high-affinity IGF-binding proteins (IGFBPs), which inhibit their interaction with the IGF-I receptor. The major binding protein in circulation is IGFBP-3[9].

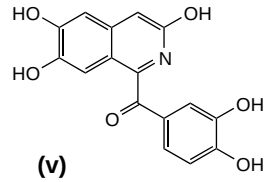
IGF-I has been evaluated in clinical trials for its ability to treat insulin-resistant diabetes and growth failure, as well as other IGF-responsive diseases. However, its peptidic nature does not allow oral availability or flexible dosing. Thus, orally active compounds that can stimulate the IGF-I receptor would have a distinct advantage over IGF-I. Despite the existence of several preliminary reports[10] on such compounds, many difficulties are predicted because the agonists would have to be able to bind both β subunits of the receptor simultaneously.

Chen and collaborators[11] have recently reported an alternative approach, which suggests the use of a small-molecule ligand able to displace bound IGF-I from the large pool of IGF–IGFBP complexes in the body. The free biologically active IGF-I could then exert its effect on responsive cells. By screening their in-house chemical libraries, they identified an initial hit compound, which was designated as L-(3,4-dihydroxyphenyl)-alanine (L-DOPA) in the library collection.

However, this result was not confirmed by an experiment using a freshly prepared sample of L-(3,4-dihydroxyphenyl)-alanine powder dissolved in DMSO. In fact, the solution showed better activity after being stirred at room temperature for three days in the air. Preparative HPLC separation of the several peaks present in the solution led to the isolation of two components that were found to actively inhibit the binding of radiolabeled IGF-I to IGFBP-3 at low-nanomolar concentrations. The compounds were fully characterized and identified as compounds **(v)** and **(vi)**. They inhibited IGF-I binding to IGFBP-3 with K_i values of 28 and 5.6 nM, respectively.

In addition, the ability of the compounds to release bioactive IGF-I from its bound form with IGFBP-3 was tested, by measuring the proliferative response of 3T3 fibroblasts expressing IGF-I receptors to IGF-I stimulation. Cell-proliferation was monitored by the quantitation of [^{3}H]-thymidine incorporation into the cells. Maximal reversal of the growth

inhibitory effects of IGFBP-3 was reached at 1 μM concentration for compound **vi**, and at 10 μM for **v**. These data are in agreement with the binding results, which show that **vi** is fivefold more potent than **v**. It should be noted that 10 μM of either compound alone had any effect on [^{3}H]-thymidine incorporation in these cells.



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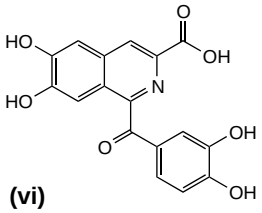


(vi)

7 Jones, J.I. *et al.* (1995) Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.* 16, 3–34

8 McInnes, C. *et al.* (1997) Growth factor receptors: structure, mechanism, and drug discovery. *Biopolymers* 43, 339–366

9 Leong, S.R. *et al.* (1992) Structure and functional expression of the acid-labile subunit of the insulin-like growth factor-binding protein complex. *Mol. Endocrinol.* 6, 870–876

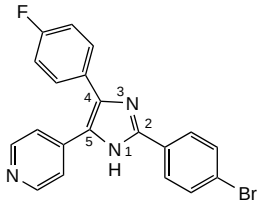
10 Zhang, B. *et al.* (1999) Discovery of a small-molecule insulin mimetic with anti-diabetic activity in mice. *Science* 284, 974–977

11 Chen, C. *et al.* (2001) Discovery of a series of non-peptide small molecules that inhibit the binding of insulin-like growth factor (IGF) to IGF-binding proteins. *J. Med. Chem.* 44, 4001–4010

Substituted imidazoles as glucagon receptor antagonists

Glucagon is a 29-amino-acid peptide hormone produced by the α-cells in the pancreas and is a major counter-regulatory hormone to insulin in the maintenance of glucose homeostasis. According to a bihormonal hypothesis, in type 2 diabetes elevated levels of circulating glucagons result in increased rates of hepatic glucose synthesis and glycogen metabolism, which leads to excessive plasma-glucose levels[12]. Therefore, antagonists of the glucagon receptor have the potential to induce a decrease in fasting plasma-glucose levels in diabetics.

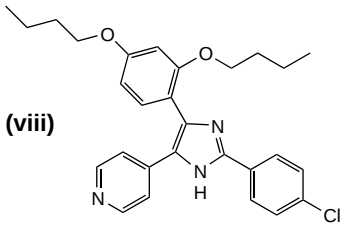
In recent years, great effort has been devoted to the synthesis of either peptidyl or non-peptidyl antagonists of glucagon[13]. Among the non-peptidyl antagonists, the triarylimidazole **(vii)** was reported to have an IC_{50} value of 0.27 μM in the human glucagon receptor (hGlur) assay. However, it also exhibited an IC_{50} value of 0.16 μM in a p38 mitogen-activated protein kinase (MAPK) assay[14].



(vii)

On this basis, Chang and coworkers have recently published their transformation of the lead compound **vii** into a potent and selective glucagon receptor antagonist[14]. A thorough SAR study at positions 1, 2 and 5 of the imidazole ring of the model **(vii)** led to the synthesis of several compounds. These were tested for their binding affinity to hGlur by measuring the reduction in binding of ^{125}I-glucagon to hGlur expressed in Chinese hamster ovary (CHO) cell membranes, in the presence of physiological concentrations of Mg^{2+} (5 mM).

In addition, the inhibition of human p38α MAPK (p38) was also assessed. Although several compounds had significant affinity for the glucagon receptor, their activity was invariably accompanied by submicromolar activity in the MAPK assay. However, separation of MAPK activity from glucagon receptor binding was achieved when the SAR of the 4-position of the imidazole was examined. The most significant compound was the 2,4-*bis*-alkoxy derivative **(viii)** (hGlur IC_{50} = 0.053 μM; p38 IC_{50} = 20% inhibition at 40 μM), which had a distinct affinity for the glucagon-receptor relative to its MAPK activity.



(viii)

Compound **viii** could provide insights into the role of glucagon receptor antagonism in the management of glucose homeostasis.

12 Unger, R.H. (1978) Role of glucagon in the pathogenesis of diabetes: the status of the controversy. *Metabolism* 27, 1691–1709

13 De Laszlo, S.E. *et al.* (1999) Potent, orally absorbed glucagon receptor antagonists. *Bioorg. Med. Chem. Lett.* 9, 641–646

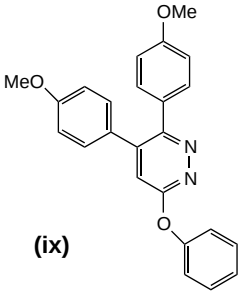
14 Chang, L.L. *et al.* (2001) Substituted imidazoles as glucagons receptor antagonists. *Bioorg. Med. Chem. Lett.* 11, 2549–2553

Pyridazinone derivatives as novel inhibitors of interleukin-1β production

Interleukin-1β (IL-1β) plays a pivotal role in the pathogenesis of inflammatory joint diseases, including rheumatoid arthritis (RA)[15]. An endogenous IL-1 receptor antagonist (IL-1Ra) was isolated and shown to be effective in animal models of RA (Ref. 16). Recently, Matsuda and collaborators reported that 3,4-*bis*(4-methoxyphenyl)-6-phenoxypyridazine **(ix)** is a potent inhibitor of IL-1β production (IC_{50} = 0.10 μM). However, the compound showed low activity *in vivo*[17]. The same group has now prepared a series of compounds **(x, a–z)**[18] that contain a pyridazinone ring with a hydrophobic substituent at the 2-position, instead of the pyridazine moiety of molecule **ix**.

The new derivatives were tested for their ability to inhibit IL-1β production in HL60 cells stimulated with lipopolysaccharide (LPS). Prednisolone (IC_{50} = 0.76 μM) was used as a reference inhibitor. SAR studies on a series of derivatives **(x, a–z)** identified several active

compounds, having IC_{50} values comparable to **ix**. However, when tested *in vivo* after oral administration to rats, many of them proved to be practically insoluble in water and showed poor oral absorption. By contrast, an olive oil solution of compound **xa** (R = 4-Cl–cinnamyl, IC_{50} = 0.10 μM), was absorbed in rats and showed strong inhibitory activity at 3 mg kg^{-1}. In addition, derivative **a** did not show any undesirable adverse effects in mice treated intraperitoneally with 30 mg kg^{-1} and was, therefore, chosen for further evaluation.



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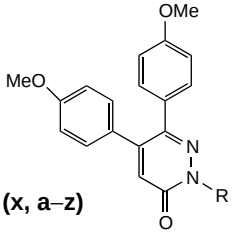


(x, a–z)

15 Gabay, C. *et al.* (2000) IL-1 inhibitors: novel agents in the treatment of rheumatoid arthritis. *Expert Opin. Invest. Drugs* 9, 113–127

16 Van den Berg, W.B. *et al.* (1994) Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment. *Clin. Exp. Immunol.* 95, 237–243

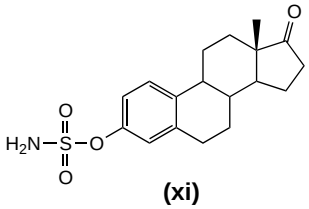
17 Matsuda, T. *et al.* (2001) Synthesis and bioactivities of novel pyridazine derivatives: Inhibitors of interleukin-1 beta (IL-1β) production. *Bioorg. Med. Chem. Lett.* 11, 2369–2372

18 Matsuda, T. *et al.* (2001) Synthesis and bioactivities of novel 5,6-*bis*(4-methoxyphenyl)-2*H*-pyridazin-3-one derivatives: Inhibitors of interleukin-1 beta (IL-1β) production. *Bioorg. Med. Chem. Lett.* 11, 2373–2375

4-Sulfamated phenyl ketone derivatives as novel inhibitors of the enzyme estrone sulfatase

Extensive research has been undertaken to identify potent and selective inhibitors of the cytochrome P450 enzyme aromatase (AR), which could be potentially useful in the treatment of hormone-dependent breast cancer[19]. However, the enzyme estrone sulfatase (ES) converts the stored (sulfated) form of the estrogens to the active (non-sulfated) form, thereby allowing the stimulation of tumors via a non-AR pathway. Therefore, several compounds have been investigated as potent inhibitors of ES, including estrone-3-sulfamate (EMATE; **xi**) and the 4-methylcoumarin-7-*O*-sulfamate derivative (COUMATE; **xii**)[20].

The observation that EMATE possesses potent estrogenic properties has led the investigation towards non-steroidal inhibitors. It is generally recognized that potent inhibitors should contain a phenolic ring and a bridging oxygen atom joining the phenyl ring to a sulfamate group[21]. Recently, Ahmed and collaborators suggested[22] that log P could also be an important factor in the inhibition process. To verify their hypothesis, they designed a project aimed to incorporate the increasing log P, while restricting the pK_a of the parent phenol. They predicted that sulfamated phenyl ketones would possess the appropriate characteristics. They synthesized and tested compounds **(xiii, 1–11)** for their ability to inhibit ES compared with EMATE (**xi**; IC_{50} = 0.5 μM; calculated log P of the parent phenol = 3.9) and COUMATE (**xii**; IC_{50} = 12 μM; calculated log P of the parent phenol = 1.7).



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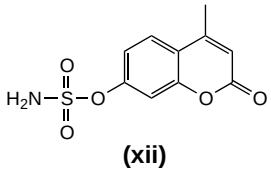
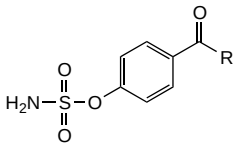


(xii)



(xiii, 1–11)

The most interesting derivatives of **xiii** were **5** (R = C_6H_{13}), **6** (R = C_7H_{15}), and **7** (R = C_8H_{17}), which were 2.4-, 2.1- and 3.5-fold more potent than COUMATE, respectively. In particular, derivative **7** (IC_{50} = 3.4 μM; calculated log P of the parent phenol = 4.1) was only 6.8 times weaker than EMATE. These derivatives were found to be irreversible inhibitors of ES. From their data the authors suggest that there is an apparent optimum log P and thus there is an alkyl chain length limit that is between 6 and 8.

In addition to these results, the weak inhibitory activity of derivative **9** (R = Ph; IC_{50} = 63 μM; calculated log P of the parent phenol = 2.9) and derivative **10** (R = CH=CHPh; IC_{50} = 263 μM; calculated log P of the parent phenol = 3.4) is attributed to the fact that the possibility for the C=O group to attract electrons from a second π system, lowers the stability of the phenoxide ion. Support for this hypothesis is provided by the fact that inhibitory activity greatly increased in derivative **11** (R = CH_2Ph; IC_{50} = 33 μM; calculated log P of the parent phenol = 2.9), where the two π systems are separated by a methylene group, which discontinues the conjugation.

These compounds are good leads in the search for more potent non-steroidal ES inhibitors.

19 Feutrie, M.L. *et al.* (1999) Aromatase inhibitors. *Bull. Cancer* 86, 821–827

20 Woo, L.W.L. *et al.* (1998) Steroidal and non-steroidal sulfamates as potent inhibitors of steroid sulfatase. *J. Med. Chem.* 41, 1068–1083

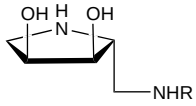
21 Purohit, A. *et al.* (1999) Recent advances in the development of steroid sulfatase inhibitors. *J. Steroid Biochem. Mol. Biol.* 69, 227–238

22 Ahmed, S. *et al.* (2001) Hydrophobicity, a physicochemical factor in the inhibition of the enzyme estrone sulfatase. *Bioorg. Med. Chem. Lett.* 11, 2525–2528

Aminomethylpyrrolidine derivatives as selective α-mannosidase inhibitors

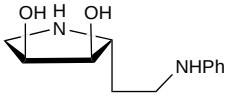
The quantitative distribution of complex carbohydrates on the surface of breast, colon and skin cancer cells is associated with disease prognosis. Thus, The specific inhibition of *N*-linked glycoprotein-processing α-mannosidases could provide a useful anticancer strategy[23]. Mannostatin A and B isolated from the soil microorganism *Streptoverticillum verticillus* and a synthetic analogue[24] are among the most potent inhibitors of α-mannosidases reported, to date. However, α-mannosidase inhibitors that are monosaccharide mimics, also inhibit other glycosidase types, in particular α-L-fucosidases[25]. It is, therefore, suggested that enzyme selectivity could be improved if the inhibitor could include some information of the glycosidic bond that is cleaved (e.g. α vs β) and of the aglycon itself. Such inhibitors could be represented by disaccharide mimics linked to monosaccharides through non-hydrolyzable linkages[26]; however, synthesis of such mimics proved to be lengthy. In addition, these compounds would not possess the essential requirements to become a drug, such as membrane permeability.

On this basis, Vogel and collaborators have recently reported on a series of (2*R*,3*R*,4*S*)-2-(substituted-amino)-methyl-3,4-dihydroxypyrrolidines (**xiv, a–k**)[27], which can mimic a transitional or intermediate structure of the hydrolytic process, and are potential novel inhibitors of α-mannosidases.



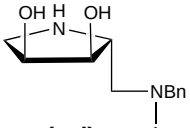
(xiv, a–k)

The compounds were tested for their activity towards 25 commercially available glycosidases. In agreement with the authors' hypothesis, which suggests that synthetic compounds could mimic a transitional or intermediate structure of the hydrolytic pathways, several compounds showed interesting properties. In particular, the most potent compound was derivative **i** (R = benzyl; K_i = 7.4 μM, competitive inhibition towards α-mannosidase purified from the plant, jack bean), which also showed the best selectivity towards α-mannosidases. It should be noted that **xv**, a structural isomer of **xiv** derivative **i** in which the amino moiety of the side chain is two-bonds apart from the pyrrolidine ring, is a much weaker α-mannosidase inhibitor (33% at 1 mM against α-mannosidase from jack bean) and is completely inactive towards the other glycosidases tested.



(xv)

This result highlights the importance of the (2*R*)-aminomethyl group of this series in a possible electrostatic interaction with a carboxylic group of the α-mannosidases. Further support for this hypothesis is given by the fact that the acetamide **(xvi)** is completely inactive.



(xvi)

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24 Ogawa, S. *et al.* (2000) Synthesis of a potent aminocyclitol α-mannosidase inhibitor, 1L-(1,2,3,5/4)-5-amino-4-*O*-methyl-1,2,3,4-cyclopentanetetrol. *Bioorg. Med. Chem. Lett.* 10, 1047–1050

25 Andersen, S.M. *et al.* (2000) Syntheses of sugar-related trihydroxyazepanes from simple carbohydrates and their activities as reversible glycosidase inhibitors. *Carbohydr. Res.* 326, 22–33

26 Fuchss, T. *et al.* (2000) 5-Amino-5-deoxy-1-thioglucopyranosides – synthesis of thioglycoside derivatives of nojirimycin. *J. Carbohydr. Chem.* 19, 677–691

27 Popowycz, F. *et al.* (2001) Derivatives of (2*R*,3*R*,4*S*)-2-aminomethylpyrrolidine-3,4-diol are selective α-mannosidase inhibitors. *Bioorg. Med. Chem. Lett.* 11, 2489–2493

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Combinatorial chemistry

Nucleoside phosphoramidates

Compounds with the phosphoramidate functionality have a range of biological activities, including acting as anticancer and cardioprotective agents. As phosphoric and carboxylic equivalents, phosphoramidates have been evaluated as analogues of nucleosides and oligonucleotides. Only a limited number of nucleoside phosphoramidates have been prepared and evaluated for antiviral activity.

A combinatorial approach was undertaken, employing screening of biologically relevant libraries for their ability to modulate biological pathways, with or without regard to specific molecular targets. This strategy is appropriate in the context of antiviral lead discovery, simultaneously identifying leads with the potential discovery of novel molecular targets[1]. A library of 600 single phosphoramidates was synthesized on solid phase. Antiviral evaluation of this library against hepatitis B virus in cell-based assays helped validate the biological relevance of the library with respect to its ability to cross cell membranes, its metabolic stability and its antiviral activity.

Several potent compounds were obtained from this library, but only preliminary activity data were presented for the library in this publication. Further lead optimization is in progress by