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

Cyclic ketone inhibitors of cathepsin K

The cathepsins are lysosomal proteases, the majority of which belong to the papain-like cysteine protease family. Most of the cathepsins have broad tissue distribution; however, cathepsin K is almost exclusively expressed in bone-resorbing osteoclasts. Specific inhibitors of cathepsin K might, therefore, have potential for the treatment of diseases involving excessive bone resorption such as osteoporosis. Recent studies involving antisense oligonucleotides and cathepsin K-deficient mice support this hypothesis.

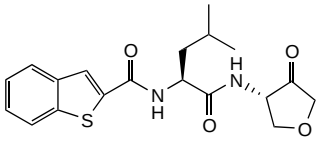
Inhibitors of cysteine proteases usually depend on an electrophilic group that forms either a reversible or irreversible covalent bond with the highly nucleophilic catalytic cysteine residue. The treatment of osteoporosis would probably require chronic administration of a therapeutic agent and thus, reversible inhibition rather than permanent covalent modification is preferred to avoid a potential immune response.

The medicinal chemistry group of GlaxoSmithKline (Harlow, UK and King of Prussia, PA, USA) has released a series of papers describing the discovery of cyclic ketone inhibitors of cathepsin K[1–3]. A library of novel cyclic alkoxy ketone inhibitors, exemplified by **(i)** (*S* isomer shown), was prepared by solid-phase synthesis, in a chiral form[1]. Molecule **(i)** was synthesized diastereoselectively and the *S*-isomer and *R*-isomer exhibited K_i values of 7 nM and 68 nM, respectively. However, the 5-membered cyclic ketones were found to be unstable to epimerization in aqueous buffer; the epimerization is presumably base-catalyzed because it is faster in HEPES buffer at pH 6.8 than in acetate buffer at pH 5.5. The 6-membered cyclic alkoxy ketones were shown to have similar potency to their 5-membered analogues, yet exhibited a much-reduced rate of epimerization[2].

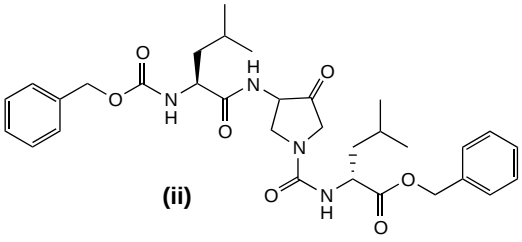
Several structures of these ketone-based inhibitors co-crystallized with cathepsin K have been described[3]. The structures are consistent with the addition of the catalytic thiol to the ketone forming a hemithioketal, with the inhibitor occupying the unprimed side of the active site.

Replacement of the ring oxygen with nitrogen allows further functionalization and the ability to span either side of the active site. This was shown to enhance binding affinity, with molecule **(ii)** exhibiting a K_i value of 0.6 nM. The incorporation of the electronegative heteroatoms was shown to dramatically increase the potency of the cyclic ketones compared with their carbocyclic analogues, which were essentially inactive.

Promising pharmacokinetics were shown for several of the compounds in this class; however, the observation that they are prone to epimerization has limited their development.



(i)



(ii)

1 Witherington, J. *et al.* (2001) Solid-phase synthesis of cyclic alkoxyketones, inhibitors of the cysteine protease cathepsin K. *Bioorg. Med. Chem. Lett.* 11, 195–198
2 Witherington, J. *et al.* (2001) Diastereoselective synthesis, activity and chiral stability of cyclic alkoxyketone inhibitors cathepsin K. *Bioorg. Med. Chem. Lett.* 11, 199–202
3 Marquis, R.W. *et al.* (2001) Cyclic ketone inhibitors of the cysteine protease cathepsin K. *J. Med. Chem.* 44, 725–736

A prodrug approach to treating hyperglycemia

Non-insulin dependent diabetes mellitus (NIDDM) is a chronic progressive metabolic disorder that accounts for 90% of all diabetes. The morbidity associated with the disease such as stroke, retinopathy

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and nephropathy, results from damage to the macro- and micro-vasculature. This damage is a result of prolonged hyperglycemia and, therefore, normalizing glucose levels is a treatment goal for NIDDM patients.

The over production of glucose by the liver contributes strongly to elevated glucose levels during fasting in NIDDM patients. The production of glucose, by the process of gluconeogenesis, is stimulated by mitochondrial oxidation of free fatty-acids. Molecule **(iii)**, SAH51641, has been shown to reduce glucose levels in animal models of diabetes by reducing fatty-acid oxidation. Molecule **(iii)** is metabolized to the active species **(iv)** by liver enzymes; the methyl group is oxidized to a carboxyl group by cytochrome P450 isoforms and, following hydrolysis of the dioxolane ring, the ketone is reduced by reductases. Molecule **(iv)** effectively sequesters coenzyme A by forming a thiol ester, thus depriving the system of an essential cofactor. However, if this process occurs in other tissues beside the liver, such as the testes, it results in toxicity.

A group at the Novartis Institute for Medical Research (Summit, NJ, USA) set out to identify a prodrug to target the formation and clearance of the active agent **(iv)** exclusively in the liver and prevent it entering the bloodstream[4]. The group evaluated a series of triglyceride derivatives, including ester and ether linkages, which led to the eventual identification of the triester prodrug **(v)**. Molecule **(v)** was found to reduce fatty-acid oxidation in both normal and diabetic animals and the active species **(iv)** was undetectable in plasma after oral dosing in normal rats (300 μmol kg^{-1}, $AUC_{0-48\ h}$ = ~0). The molecule provided significant glucose lowering in the chronic STZ diabetic rat model (ED_{50} = 39 mg kg^{-1} day^{-1}) and a 28-day safety study did not generate any observable histological effects.

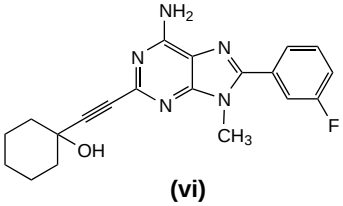
Thus, the triester **(v)** appears to have met the requirements of: (1) being absorbed into the liver intact, (2) being metabolized to the active agent at an appropriate rate so as to give the desired pharmacological effect, but not overload the metabolism, and (3) being efficiently cleared. Molecule **(v)** has thus been selected as a development candidate.

4 Bebernitz, G.R. *et al.* (2001) Reduction in glucose levels in STZ diabetic rats by 4-(2,2-dimethyl-1-oxopropyl)benzoic acid: a prodrug approach for targeting the liver. *J. Med. Chem.* 44, 512–523

Adenosine receptor antagonists as a potential treatment of hyperglycemia

The main cause of morbidity in patients with NIDDM is prolonged elevated levels of glucose, leading to disease of the vasculature. As described above, the increased production of glucose by the liver is a major factor in hyperglycemia. Adenosine is involved in the regulation of glucose levels and it has been shown that adenosine agonists, selective for the A_2 receptor, stimulate hepatic glucose production. A group at Tsukuba research laboratories (Ibaraki, Japan) set out to identify a selective A_2 antagonist as a novel class of antidiabetic agent[5].

9-Methyl adenine is a known adenosine antagonist and substitution at position 2 is known to impart potency and selectivity for the A_2 receptor. 8-Aryl substitution was found to increase activity and the group decided to combine these structural features and prepared a tri-substituted adenine library exemplified by molecule **(vi)**. The series of molecules was evaluated for effect on glucose production in rat hepatocytes, induced by a non-selective adenosine agonist (*N*-ethylcarboxamidoadenosine, NECA). Molecule **(vi)** inhibited NECA-induced glucose production with a K_i value of 0.46 μM. No effect on basal glucose production was observed for **(vi)** in the absence of NECA, showing that it lacked partial agonist activity.



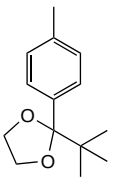
(vi)

In a Chinese hamster ovary (CHO) cell line stably transfected with the human A_{2B} receptor, molecule **(vi)** inhibited NECA-induced stimulation of cAMP production with a K_i value of 0.063 μM. This inhibitory effect was much more potent using the non-selective agonist NECA than for agonists selective for the A_1, A_{2A} and A_3 receptor subtypes. These results are consistent with the hypothesis that adenosine stimulates glucose production via the A_{2B} receptor.

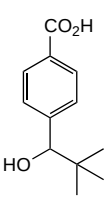
Furthermore, the adenine derivative **(vi)** was found to reduce blood-glucose levels in a dose-dependent manner in a mouse model of diabetes (KK-A^y mouse) upon oral administration (10 and 30 mg kg^{-1}).

Molecule **(vi)** is a non-selective adenosine antagonist (K_i = 0.02 μM for A_1, 0.01 μM for A_{2A} and 1.1 μM for A_3) and efforts are ongoing to identify a selective A_{2B} antagonist as a potential new approach to treating hyperglycemia in NIDDM patients.

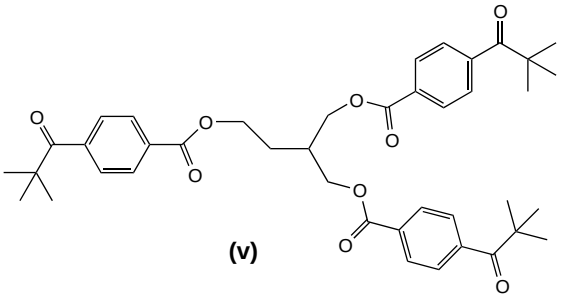
5 Harada, H. *et al.* (2001) 2-Alkynyl-8-aryl-9-methyladenines as novel adenosine receptor antagonists: their synthesis and structure–activity relationships toward hepatic glucose production induced via agonism of the A_{2B} receptor. *J. Med. Chem.* 44, 170–179



(iii)



(iv)

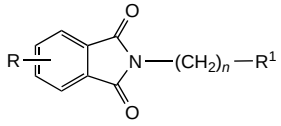


(v)

Novel α-glucosidase inhibitors

The enzyme α-glucosidase (EC 3.2.1.20) catalyzes the final step in the digestive processing of carbohydrates. Therefore, its inhibitors have potential in the management of diseases such as diabetes and certain forms of hyperlipoproteinemia, as well as in the treatment of obesity. They are also considered as potential antiviral and anticancer agents. Recently their use in the treatment of AIDS has been suggested.

During studies on a series of tumor necrosis factor-α (TNF-α) production regulators, a Japanese group identified potent agents with a phthalimide skeleton **(vii)**[6]. Some of them showed the expected hypoglycemic activity; this, however, did not correlate with their TNF-α production-regulating activity. They hypothesised that α-glucosidase inhibition could be involved. Therefore, all the compounds were tested in comparison with 1-deoxynojirimycin (dNM), a well known α-glucosidase inhibitor[7]. Some of these compounds, which had a tetrachlorosubstituted phthalimide moiety, displayed activity higher than the model by one or two orders of magnitude. Their IC_{50} values ranged from 0.7–2.6 μM.



(vii)

This new series could represent an innovative approach to the treatment of various diseases as well as to mechanistic studies of α-glucosidase inhibition.

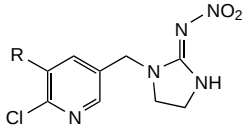
6 Takahashi, H. *et al.* (2000) α-Glucosidase inhibitors with a phthalimide skeleton: structure–activity relationship study. *Chem. Pharm. Bull.* 48, 1494–1499

7 Inouye, S. *et al.* (1968) Structure and synthesis of nojirimycin. *Tetrahedron* 24, 2125–2144

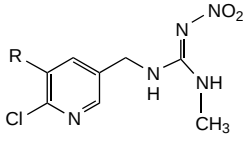
Azidopyridinyl compounds as candidate photoaffinity probes for nicotinic acetylcholine receptors

The most commonly used photoaffinity probes for the structural analysis of nicotinic acetylcholine receptors (nAChRs) include [^{3}H]nicotine, [^{3}H]5-azidonicotine and a 2-azido-5-^{125}iodobenzoyloxyethyl derivative of an imidacloprid analogue[8]. In particular, the nicotine derivatives are selective for mammal nAChRs and the imidacloprid analogues for insect nAChRs. Therefore, photoaffinity ligands that have nanomolar potency on both mammalian and insect nAChRs would be highly desirable.

Based on the consideration that aryl azides are the most popular photoaffinity reagents, Casida and colleagues devised the 5-azido-6-chloropyridin-3-yl substituent as a suitable candidate for such a probe[9]. Compound **(viii)** was synthesized as the azido derivative of imidacloprid **(ix),** and **(x)** as the azido derivative of its open analogue **(xi)**. Their effects were evaluated using rat brain, rat $\alpha_4\beta_2$ integrin, *Myzus* and *Drosophila* membrane preparations. Although the model compounds **(ix)** and **(xi)** have relatively poor affinities in rat brain membranes and in $\alpha_4\beta_2$ preparation, the azido substituent increases the affinity [**(viii)** and **(x)** = 44 nM with brain and **(x)** = 4.4 nM with $\alpha_4\beta_2$ membranes). All the compounds have high affinities for the insect preparations (K_i values = 1–15 nM). Therefore, compounds **(viii)** and **(x)** offer the possibility of using the same photoprobe for mammals and insects when evaluating the structural basis for selectivity.



(viii) R = N_3
(ix) R = H



(x) R = N_3
(xi) R = H

8 Tomizawa, M. *et al.* (1997) [^{125}]Azidinicotinoic photoaffinity labeling of insecticide-binding subunit of *Drosophila* nicotinic acethylcholine receptor. *Neurosci. Lett.* 237, 61–64

9 Kagabu, S. *et al.* (2000) 5-Azidoimidacloprid and an acyclic analogue as candidate photoaffinity probes for mammalian and insect nicotinic acetylcholine receptors. *J. Med. Chem.* 43, 5003–5009

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Combinatorial chemistry

Neuroimmunophilin ligands

Recently, several groups have disclosed novel compounds derived from the peptidylprolyl isomerase (PPIase)-inhibition domains of the immunosuppressive drugs FK506 and rapamycin. These non-immunosuppressive small molecules, with a MW of <500, were found to be potent neurotrophic agents in neurite outgrowth assays. Certain analogues, such as GPI1046 **(i)** have also been shown to be effective in models of Parkinson's and Alzheimer's diseases. Dosing paradigms have shown that these compounds cause both neuroprotective and neuroregenerative effects in models of CNS pathogenicity.

The biological target for the exceptional neurotrophic activity of compounds such as **(i)** is not known, although FKBP-12 is a probable candidate because it is upregulated in models of CNS injury. It has been shown, however, that there is no clear correlation between rotamase inhibition activity and the ED_{50} value for neurite outgrowth. In an effort to produce SARs in one compound series,

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