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.

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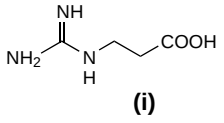
Molecules

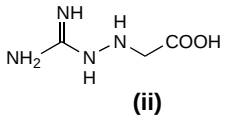
Novel amino- and diaminoguanidinoacetic acid derivatives as antidiabetic agents

Type-2 diabetes is a metabolic disorder characterized by resistance of the peripheral target tissues to fully respond to the binding of insulin, and also, insufficient insulin secretion by the pancreas to overcome this reduced response. This results in impaired glucose uptake and metabolism and, eventually, to fasting hyperglycemia.

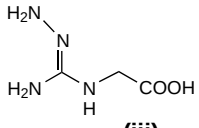
Recently, the 3-guanidinopropionic acid **(i)** was reported to have both antihyperglycemic and antiobesity activity in the KKA^y mouse, a rodent model of type-2 diabetes[1]. However, besides a relatively low potency, **(i)** has a significant propensity to accumulate in muscle. On these bases, a group at Pharmacia Corporation[2] (Kalamazoo, MI, USA) undertook a programme to identify novel, more potent analogues, which lacked this side effect. Although **(i)** proved to tolerate very few modifications, the aminoguanidinoacetic acid analogue **(ii)** showed equipotent antidiabetic activity and was less prone to accumulate in muscle. In a mouse insulin sensitizing screen nonfasting blood glucose levels, the test/control values (MISS T/C) were 0.52 ± 0.24 and 0.61 ± 0.29 for compounds **(i)** and **(ii)**, respectively. Therefore, compound **(ii)** became the new lead. An exhaustive SAR was undertaken, which confirmed that, as with compound **(i)**, compound **(ii)** had a high intolerance to structural modifications. However, the isomeric aminoguanidine [**(iii)**, MISS T/C = 0.51 ± 0.24] and the diaminoguanidine [**(iv)**, MISS T/C = 0.69 ± 0.22] were found to retain antidiabetic activity, when tested against compounds **(i)** and **(ii)** in the models of obese hyperglycemic, hyperinsulinemic, insulin resistant KKA^y mice[3].



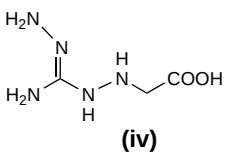
(i)



(ii)

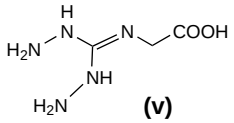


(iii)

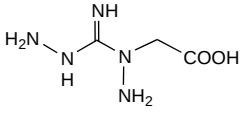


(iv)

Several pieces of evidence from this SAR seem to indicate that the terminal amino group on the guanidine moiety is a key component of the antidiabetic activity of this class. Further studies using both compounds **(iii)** and **(iv)** did not enable the identification of any improved aminoguanidine derivative, whereas in the case of the diaminoguanidine substrate, compounds **(v)** (MISS T/C = 0.23 ± 0.05) and **(vi)** (MISS T/C = 0.43 ± 0.20) were found to be more active than compound **(iv)**. Because compound **(i)** was known to accumulate in muscle tissue owing to it being a substrate for both the creatine transporter and creatine kinase, compounds **(v)** and **(vi)** were also tested as substrates for the two enzymes. Both enzymes proved to be able to discriminate between compound **(i)** and derivatives **(v)** and **(vi)**. This suggests that the latter compounds are unlikely to accumulate in the muscle and, therefore, could become possible candidates for the treatment of type-2 diabetes.



(v)



(vi)

1 Meglasson, M.D. *et al.* (1993) Antihyperglycemic action of guanidinoalkanoic acids: 3-guanidinopropionic acid ameliorates

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hyperglycemia in diabetic KKA^{y} and C57BL6J *ob/ob* mice and increases glucose disappearance in Rhesus monkeys. *J. Pharmacol. Exp. Ther.* 266, 1454–1462

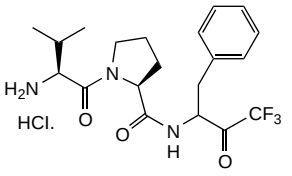
2 Larsen, S.D. *et al.* (2001) Synthesis and biological activity of analogues of the antidiabetic/antiobesity agent 3-guanidinopropionic acid: discovery of a novel aminoguanidinoacetic acid antidiabetic agent. *J. Med. Chem.* 44, 1217–1230

3 Vaillancourt, V.A. *et al.* (2001) Synthesis and biological activity of aminoguanidine and diaminoguanidine analogues of the antidiabetic/antiobesity agent 3-guanidinopropionic acid. *J. Med. Chem.* 44, 1231–1248

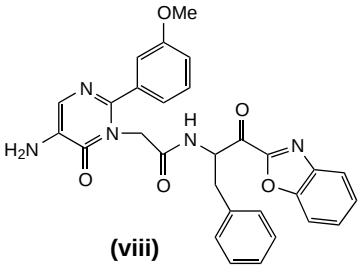
Non-peptidic inhibitors of human chymase

Chymase is a serine protease of the chymotrypsin family that is secreted from mast cells during an immunological response. The natural substrates of chymase have not yet been identified and its precise physiological function is unknown. However, because of its localization to mast cells it is believed that inhibitors might have a therapeutic effect in inflammatory diseases. The drug discovery group at the Welfide Corporation (Osaka, Japan) set out to discover selective, metabolically stable inhibitors of chymase to further characterize its pathophysiological role[4,5].

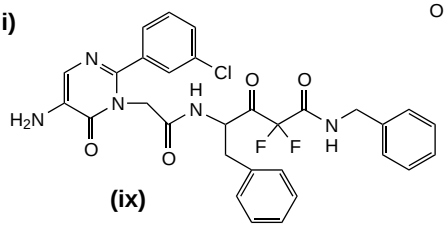
Several peptidic inhibitors of chymase are known, exemplified by compound **(vii)**, containing an electrophilic group designed to form a reversible covalent bond with the catalytic serine of the enzyme. In an effort to identify nonpeptidic inhibitors, a pyrimidinone moiety was incorporated as a P_3-P_2 mimetic. Optimization was conducted on two different series of electron deficient carbonyl groups as the electrophile: α-ketoheterocycles[4] or difluoromethylene ketones[5], exemplified by compounds **(viii)** and **(ix)**, respectively. Both compounds are potent and selective inhibitors of chymase: compound **(viii)** has K_i values of 4.8 nM and 943 nM for chymase and chymotrypsin, respectively, and compound **(ix)** has K_i values of 2.6 nM and 458 nM for chymase and chymotrypsin, respectively. These compounds are orally bioavailable at 19% for **(viii)** and 17% for **(ix)** in the rat. Compound **(viii)** has a long half-life (35 h) and although the half-life for **(ix)** is much shorter (2.1 h), it does exhibit a rapid high maximum plasma concentration (1.5 μg ml^{-1}) after oral administration.



(vii)



(viii)



(ix)

The biological profiles of these two molecules has warranted them being put forward for further studies to elucidate the pathophysiological role of chymase.

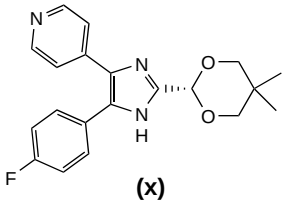
4 Akahoshi, F. *et al.* (2001) Synthesis, structure–activity relationships and pharmacokinetic profiles of nonpeptidic α-keto heterocycles as novel inhibitors of human chymase. *J. Med. Chem.* 44, 1286–1296

5 Akahoshi, F. *et al.* (2001) Synthesis, structure–activity relationships and pharmacokinetic profiles of nonpeptidic difluoromethylene ketones as novel inhibitors of human chymase. *J. Med. Chem.* 44, 1297–1304

p38 MAP kinase inhibitors for the treatment of arthritis

Rheumatoid arthritis is a chronic inflammatory disease whose treatment is, at present, frequently insufficient to prevent disease progression. Two inflammatory cytokines, interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α), are important in the initiation and progression of the disease. The release of these cytokines and the response of the cells to them are both regulated by a phosphorylation cascade involving mitogen-activated protein kinases (MAPKs) including p38. Selective inhibitors of p38 MAPK have been shown to reduce the release of IL-1β and TNF-α from phagocytes and inhibit the actions of the cytokines in various inflammatory cells. p38 MAPK is thus an important target for inflammatory diseases, such as rheumatoid arthritis.

A group at Aventis (Dagenham, UK) recognized the similarity of their proprietary series of diaryl substituted 2-dioxanylimidazoles, originally developed as ACAT (acyl-CoA cholesterol *O*-acyl transferase) inhibitors, to known p38 inhibitors[6]. The substitution of a phenyl group for a pyridine group facilitated the formation of a key hydrogen bond to the kinase, and converted the series from ACAT inhibitors to p38 MAPK inhibitors, represented by compound **(x)** with an IC50 value for p38 MAPK of 0.8 μM. Deficiencies in some of the known p38 MAPK inhibitors were found to include toxicity associated with mutagenicity and CYP1A1 induction.



(x)

Optimization of this series of compounds was conducted by parallel synthesis following a screening cascade of potency, mutagenicity and CYP1A1 induction. Molecule **(xi)** was identified as a potent inhibitor of p38 MAPK (with an IC_{50} value of 0.05 μM) that also inhibits TNF-α release *in vitro* (EC_{50} = 0.11 μM, from

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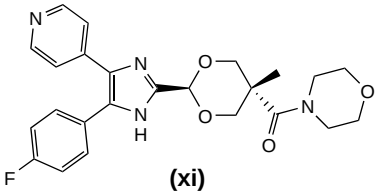
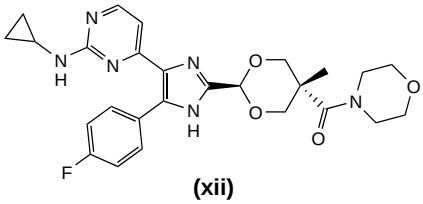
human monocytes) and *in vivo* (ED_{50} = 6 mg kg^{-1} in mice). The molecule has proven to be chemically stable and highly water soluble as the mesylate salt and has been chosen as a development candidate for the treatment of rheumatoid arthritis.



(xi)



(xii)

A back-up candidate, in which the pyridine group has been replaced by a pyrimidine [compound **(xii)**], was found to be more potent *in vitro* (IC_{50} = 0.009 μM and EC_{50} = 0.06 μM in a whole cell assay) than **(xi)**[7]. Compound **(xii)** was also found to have a decreased inhibition of cytochrome P450 enzymes compared with compound **(x)**.

6 McLay, I.M. *et al.* (2001) The discovery of RPR200765A, a p38 MAP kinase inhibitor displaying a good oral anti-arthritic efficacy. *Bioorg. Med. Chem.* 9, 537–554

7 Halley, F. *et al.* (2001) RPR 203494, a pyrimidine analogue of the p38 inhibitor RPR200765A with improved *in vitro* potency. *Bioorg. Med. Chem. Lett.* 11, 693–696

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Drug delivery

An oral delivery system for insulin

Insulin is indispensable in the treatment of patients with type-1 diabetes. Unfortunately, the discomfort of multiple daily injections lowers compliance, and most patients express the desire for a more convenient and acceptable route of administration. Among the routes being explored, the oral route of administration is the most preferred. In addition to the convenience and higher compliance with oral administration, this method would place the drug in the portal circulation first, thus mimicking the physiological pathway of insulin delivery, providing a direct route to the active site (liver) and avoiding some of the undesirable peripheral effects observed when insulin is injected. However, the oral bioavailability of insulin, as with all protein drugs, is extremely low owing to absorption barriers and enzymatic degradation in the gastrointestinal (GI) tract.

One approach for oral formulations of peptide drugs has been to combine the drug with enzyme inhibitors[1–3], but this approach has met with limited success. In some cases, the enzyme inhibitors have not only failed to significantly increase the oral bioavailability of the drug but also they disturb the digestion of nutritive proteins and cause undesirable effects on the pancreas[1–3]. Therefore, this approach is not practical unless these side effects can be avoided. Another strategy involves the use of drug delivery systems with mucoadhesive properties. Compounds can be covalently attached to these mucoadhesive polymers, thereby producing conjugates that prolong the residence time of the compound in the intestine, while reducing undesirable systemic side effects by keeping the peripheral concentration of the compounds at low levels. Mucoadhesive polymer–enzyme inhibitor conjugates could, therefore, potentially reduce the undesirable side effects of inhibitors in drug delivery systems.

Using extended mucoadhesive polymer–insulin conjugates

Recently, Marschütz and colleagues have reported the application of a strategy in which enzyme inhibitors are covalently attached to a mucoadhesive polymer and combined with insulin, as well as a second mucoadhesive polymer–cysteine conjugate, to produce a formulation that orally delivers insulin in diabetic rats[4]. The strategy takes advantage of several aspects of the various components within the drug delivery system: (1) prolonged residence time of polymer–inhibitor conjugates; (2) avoidance of undesirable systemic side effects of the inhibitors by keeping them concentrated on the polymer conjugate; (3) the use of a polymer–cysteine conjugate, which has been shown to protect the insulin within the tablet from enzymatic degradation and allow for a prolonged controlled release from the tablet; and (4) the use of insulin that is not modified itself and so no prodrug metabolism is necessary.

Polymer–inhibitor conjugates were produced by condensing the polymer carboxymethylcellulose (CMC) with the enzyme inhibitors elastatinal (Ela) and Bowman-Birk inhibitor (BBI). The authors had previously shown, in *in vitro* studies, that these CMC–BBI and CMC–Ela conjugates effectively inhibit the peptidases trypsin, chymotrypsin and elastase. Similarly, a conjugate of polycarbophil (PCP) and cysteine (Cys) was made. Previously, this relatively new PCP–Cys mucoadhesive polymer had been shown to exhibit a much longer residence time in the intestinal mucosa (up to 10 h) than polycarbophil itself[5–7]. When formulated with model drugs, the resulting residence times have also been long.

Insulin dosage forms were prepared by compressing tablets consisting of insulin, CMC–BBI, CMC–Ela, PCP–Cys and mannitol. Appropriate control formulations without enzyme inhibitors and without insulin were also prepared for

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