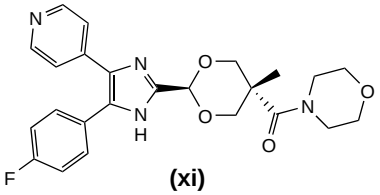
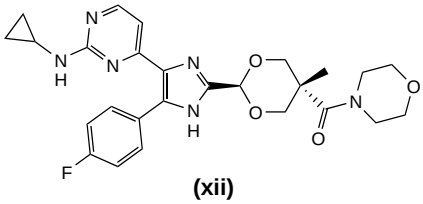
human monocytes) and *in vivo* ($ED_{50}$ = 6 mg $kg^{-1}$ in mice). The molecule has proven to be chemically stable and highly water soluble as the mesylate salt and has been chosen as a development candidate for the treatment of rheumatoid arthritis.



**(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A back-up candidate, in which the pyridine group has been replaced by a pyrimidine [compound **(xii)**], was found to be more potent *in vitro* ($IC_{50}$ = 0.009 μM and $EC_{50}$ = 0.06 μM in a whole cell assay) than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)**.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**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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comparison. Insulin is degraded in the GI tract by several enzymes, including trypsin, chymotrypsin and elastase. The protective effect of the delivery system towards enzymatic attack of insulin by these three enzymes was evaluated *in vitro*. In these studies, the incorporated insulin was almost completely degraded in the dosage form without CMC–BBI and CMC–Ela, whereas ~50% of the insulin remained intact in the delivery system containing the polymer–inhibitor conjugates[4]. The release rate of insulin from the dosage form was also studied *in vitro* and a slow release was observed in artificial intestinal fluid (pH 7.1).

To determine the efficacy of the insulin delivery system, an *in vivo* study was performed in which diabetic Balb/C mice were dosed with the insulin drug delivery system tablets[4]. Even though the CMC–BBI and CMC–Ela inhibitor conjugates only account for 20% of these drug delivery systems, the bioavailability of orally administered insulin from this formulation was significantly improved. Basal glucose levels of diabetic mice were reduced by 20–40% when dosed with the insulin drug delivery system. This effect appears 4 h after administration and is maintained for ~80 h, then gradually returns to initial values. In comparison, tablets without insulin had no effect on the blood glucose level, indicating that the effect is not caused by the CMC–BBI/CMC–Ela formulation itself. Oral administration of insulin in aqueous ascorbic acid solution also had no influence on blood glucose levels.

### Mechanism of action

There are presumably several reasons that this drug delivery system increases the oral bioavailability of insulin. Luminally secreted enzymes must penetrate the polymeric network of the tablet to degrade the embedded insulin. The PCP–cysteine conjugate is capable of forming intermolecular as well as intramolecular disulfide bonds within the polymeric network, thereby contributing stability to the dosage form. The reactive thiol groups of PCP–Cys contribute to a mucoadhesive effect that is twice that of PCP itself, presumably from thiol or disulfide exchange reactions with cysteine-rich mucin glycoproteins. The high stability of the carrier matrix allows for a prolonged release of the drug, over ~10 h *in vitro*. Once released, the inhibitory effects of CMC–BBI and CMC–Ela also provide the insulin with some protection from the action of proteolytic enzymes. Of course, the therapeutic window for insulin is narrow and rodent studies do not always extend well to human patients, but this could prove to be a good starting point for an oral insulin delivery system.


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## Combinatorial chemistry

### Philanthotoxin analogues

Philanthotoxins, a group of non-competitive antagonists of ionotropic receptors, are composed of long-chain polyamines connected to a relatively nonpolar headgroup via an amide bond. Recently, interest in the medicinal chemistry and pharmacology of philanthotoxins has been highlighted by the observation that the specificity of their antagonist action on various classes of ionotropic receptors can be achieved by modification of the polyamine portion of the molecule. Thus, natural and synthetic toxins **(i)** are known to antagonize various types of nicotinic acetylcholine receptors (nAChRs) and ionotrophic glutamate receptors (iGluRs) with similar potency. However, analogues in which the secondary amino groups are replaced by methylene groups or oxygen atoms **(ii)**, exhibit enhanced antagonist activity on mammalian muscle-type nAChR and Torpedo nAChR but are inactive on several types of iGluR.

Previous structure–activity investigations of synthetic analogues containing a symmetrical spermine moiety or closely related polyamine, which have been tested on iGluR and nAChR, emphasize the importance of the hydrophobic character of the headgroup. By contrast, no information regarding the influence of the structure of the headgroup on the potency of philanthotoxin analogues lacking inner basic sites is available.

In an effort to produce SARs in such series, a library of compounds was synthesized to test whether compounds that lack the inner basic sites bind to nAChR in a similar fashion[1]. A library of 18 individual compounds was synthesized on trityl chloride solid phase. Of those compounds tested that lacked the inner basic sites, all were inactive when tested on rat brain non-NMDA receptors. The success of this library protocol lies in increasing the understanding of the SAR of antagonism of nAChR by


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