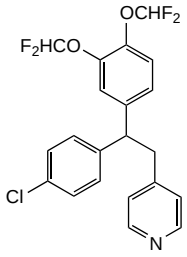
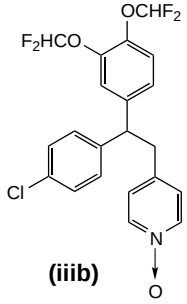
studies directed towards improving the potency and metabolic stability of CDP840. CDP840 suffers from extensive metabolism *in vitro* [8] and this translates into a short half-life *in vivo*. In the rat, the major metabolic pathway of CDP840 is the *para*-hydroxylation on the pendant group, whereas in human hepatocytes the major metabolite is the pyridinium glucuronide, which is not detected in rat hepatocytes.

The goal of this project was to increase both the potency and the metabolic stability of CDP840. After trying many structural modifications of the cathecol ring, which led to a constant loss of potency, the group obtained a significant improvement with the *bis*-difluoromethoxy analogue of CDP840 (compound **iiia**) and its corresponding *N*-oxide (compound **iiib**), which showed an increased potency for the inhibition of LPS-induced TNF-α in human whole blood (IC_{50} = 4.5 μM, 3.8 μM and 16 μM for **iiia**, **iiib**, and CDP840, respectively).

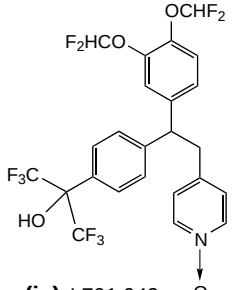


(iiia)



(iiib)

Having discovered the metabolically stable cathecol, the group focused on potency. They synthesized a few hundred analogues in this series, which showed that substitution in the *para*-position was in general better tolerated. Compound L791,943 (**iv**) was selected for *in vitro* evaluation of its metabolism in rat hepatocytes and compared with CDP840. The results indicated that, in standard incubation conditions, >98% of the parent drug remained in the case of L791,943 whereas only 11% of CDP840 was left intact.



(iv) L791,943

Additional assays showed that L791,493 was active in blocking the ovalbumin-induced bronchoconstriction in conscious guinea pig by 58% at a dose of 1 mg kg^{-1} (intraperitoneally 4 h pretreatment). L791,493 showed good *in vivo* activity in the anesthetized squirrel monkey and in the conscious sheep models of ascaris-induced bronchoconstriction. Finally, the potential of L791,943 for causing hemesis was assessed. Ferrets could be dosed orally up to 30 mg kg^{-1}, with plasma concentrations reaching 14 μM, without causing emesis.

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7 Guay, D. *et al.* (2002) Discovery of L-791,943: A potent, selective, non emetic and orally active phosphodiesterase-4 inhibitor. *Bioorg. Med. Chem. Lett.* 12, 1457–1461

8 Li, C. *et al.* (2001) Investigation of the *in vitro* metabolism profile of a phosphodiesterase-IV inhibitor, CDP840: leading to structural optimisation. *Drug Metab. Dispos.* 29, 232–241

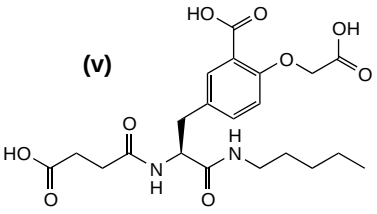
Daniela Barlocco
University of Milan
Viale Abruzzi 42
Milano 20131, Italy
tel: +39 02 5031 7515
fax: +39 02 5031 57565
e-mail: daniela.barlocco@unimi.it

Identification of a protein tyrosine phosphatase 1B inhibitor with cellular activity

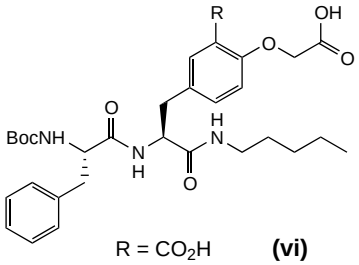
A characteristic of non-insulin dependent diabetes mellitus (NIDDM) is the resistance of cells to insulin. The cellular response to insulin binding to its receptor is dependent upon the auto-phosphorylation of the insulin receptor on a tyrosine residue and the subsequent phosphorylation of downstream effector proteins. In insulin-resistant cells the signalling cascade is attenuated most probably as a result of a defect in the insulin receptor itself.

Protein tyrosine phosphates (PTPs) in conjunction with protein tyrosine kinases (PTKs) regulate the level of protein phosphorylation. The protein tyrosine phosphate (PTP1B) has been shown to negatively regulate insulin signalling and thus inhibitors of PTP1B would be expected to prolong the activated state of the insulin receptor and be a potential novel treatment for NIDDM.

Most progress towards specific phosphatase inhibitors has been to mimic the natural substrate; a phosphotyrosine containing polypeptide. As part of a collaboration between scientists at Biovitrum (http://www.biovitrum.com) and Pharmacia (http://www.pharmacia.com), the peptide Ac-NH-Asp-Tyr(SO_3H)-Nle-NH_2 was identified as a competitive inhibitor of PTP1B (K_i = 5 μM) [9].



(v)



R = CO_2H (vi)

R =  (vii)

The discovery of the *O*-carboxymethyl salicylic acid moiety as an effective phosphotyrosine mimic led to the identification of compound **v** (K_i = 2 μM). However, it has proven difficult to achieve cell permeability for PTP inhibitors and thus the groups focussed on replacing one or more of the carboxyl groups.

The succinate group was replaced by a phenylalanine derivative to give the dipeptide **vi** (K_i = 5 μM) with a neutral N-terminus. The preparation of various analogues at the tyrosine head group highlighted the importance of acidic functionalities at these positions. The tetrazole analogue **vii** was equipotent to **vi** and found to have significantly higher Caco-2 cell permeability, although still lower, than previously reported compounds (P_{app} a → b = 1.9 ± 1.4 × 10^{-7} cm sec^{-1}, P_{app} b → a = 5.1 ± 0.3 × 10^{-7} cm s^{-1}).

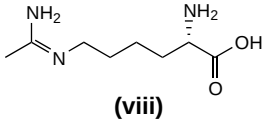
Compound vii was also shown to have modest activity at augmenting insulin stimulated uptake of 2-deoxyglucose by intact L6 myocytes. This is the first reported positive cell activity data from a non-prodrug PTP inhibitor.

9 Liljebris, C. (2002) Investigation of potential bioisosteric replacements for the carboxyl groups of peptidominietic inhibitors of protein tyrosine phosphatase 1B: identification of a tetrazole-containing inhibitor with cellular activity. *J. Med. Chem.* 45, 1785–1798

A prodrug of the selective iNOS inhibitor L-NIL

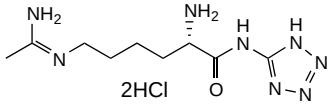
Nitric oxide is a potent cell signalling agent and is synthesized by three distinct isoforms of nitric oxide synthase. Two of these enzymes are constitutive (eNOS predominant in the endothelium and nNOS mainly in the nervous system) and generate low levels of NO, which is involved in the regulation of many physiological processes including blood pressure and neurotransmission. The third isoform, iNOS, is induced by endotoxin and cytokines and is responsible for the generation of high sustained levels of NO leading to cellular toxicity and tissue damage.

The selective iNOS inhibitor L-*N*6-1-imminoethyl lysine (L-NIL; **viii**) has been shown to have *in vivo* efficacy when administered orally at reducing paw swelling associated with the overproduction of NO in animal models of acute and chronic inflammation. Significantly L-NIL did not cause an increase in systemic blood pressure at efficacious doses indicating *in vivo* selectivity. However, the pharmaceutical properties of L-NIL are sub optimal because it is rapidly deliquescent upon exposure to air.



(viii)

A group from the Department of Inflammation and Arthritis, Pharmacia Corporation, set out to identify a prodrug of L-NIL that possesses the necessary physical properties [10]. The tetrazole-amide **ix** has no inherent inhibitory activity for the various NOS isoforms; however, it is rapidly converted to L-NIL and amino-tetrozole *in vivo*. Compound **ix** demonstrated similar dose response levels to L-NIL in both acute and chronic inflammatory models. For example, in the rat adjuvant induced arthritis model, prophylactic treatment with **ix** gave complete inhibition of paw swelling at doses between 6 and 20 mg kg^{-1} day^{-1}.



(ix)

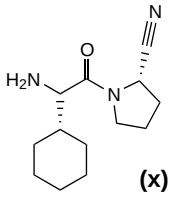
Compound **ix** is a stable, non-hygroscopic, highly water soluble crystalline solid that is being further explored in preclinical and clinical settings.

10 Hallinan, E.A. (2002) Synthesis and biological characterisation of L-*N*6-(1-iminoethyl)lysine 5-tetrazole-amide, a prodrug of a selective iNOS inhibitor. *J. Med. Chem.* 45, 1686–1689

Orally bioavailable dipeptidyl peptidase IV inhibitor

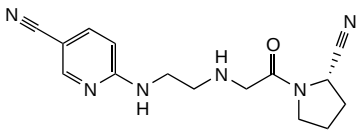
The serine protease dipeptidyl peptidase IV (DPP-IV) is a ubiquitous enzyme that cleaves N-terminal dipeptides from polypeptides with proline or alanine as the penultimate residue. DPP-IV plays a main role in the inactivation of glucagon-like peptide (GLP-1); the most insulinotropic hormone known, to date. An inhibitor of DPP-IV might be of value in treating type 2 diabetes because it would augment the action of GLP-1, giving rise to the stimulation of insulin secretion, inhibition of the release of glucagon and the slowing of gastric emptying.

The known inhibitors of DPP-IV resemble a dipeptide containing an electrophile to interact with the catalytic serine and a proline mimic at the P-1 site. A notable example is compound **x** where the electrophilic group, being a nitrile, is relatively mild and this compound is both potent (IC_{50} = 1.4 nM), selective and orally bioavailable.



(x)

A group from the Novartis Institute of Biomedical Research (http://www.novartis.com) noticed that *N*-methyl glycine was tolerated in the P-2 site. They prepared a



(xi)

library of 2-pyrrolidinecarbonitriles with a diverse range of *N*-substituted glycines using solid- and solution-phase techniques. Compound **xi** was identified as a highly potent (IC_{50} = 7 and 22 nM against human plasma and epithelial cell surface DPP-IV, respectively), selective over related peptidases and exhibiting good specificity when profiled against a panel of enzymes and receptors. Molecule **xi** is reasonably stable; the rate of cyclization to the inactive six-membered amidine is slow ($t_{1/2}$ = >48 h) at neutral pH.

Oral administration of **xi** (1 mmol kg^{-1}) reduced plasma glucose levels 38% in the cynomolgous monkey in a glucose tolerance test. In humans an oral 100 mg dose showed >80% inhibition of plasma DPP-IV activity for ~4 h and a significant increase in GLP-1 levels. Although it exhibits a relatively short half-life (0.85 h) the compound has been selected for further study in clinical trials for type 2 diabetes.

3 Villhauer, E.B. (2002) 1-[2-[(5-cyanopyridin-2-yl)amino]-ethylamino]acetyl-2-(s)-pyrrolidine-carbonitrile: A potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J. Med. Chem.* 45, 2362–2365

Steven Langston
Millennium Pharmaceuticals
Merrifield Centre, Rosemary Lane
Cambridge, UK CB1 3LQ
tel: +44 1223 722400
e-mail: steve.langston@mpi.com

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