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The design and synthesis of selective inhibitors for the insulin tyrosine kinase receptor

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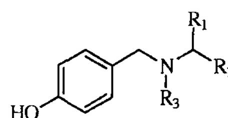
The insulin receptor is a protein tyrosine kinase (PTK), comprising two extracellular ligand-binding α domains disulphide-linked to two β-domains which traverse the plasma membrane and carry the kinase domains (Ullrich & Schlessinger, 1990). Binding of insulin to the α-subunits, through a change in the quaternary structure of the receptor, enables ATP binding and subsequent autophosphorylation of specific tyrosines (Y1158, Y1162 and Y1163) in the activation loop of the kinase domain (Hubbard et al. 1994). As a result, kinase activity is stimulated towards exogenous substrates, initiating signal transduction and maintaining the PTK in the activated state (White et al., 1988).

Specifically directed inhibitors of insulin receptor kinase (IRK) would be useful tools in determining the relationship between autophosphorylation and kinase activity with the various biochemical pathways in response to insulin signal transduction. (Groundwater et al., 1996; Schecter et al. 1989). The X-ray crystal structure of the unphosphorylated apo PTK domain of the human IRK has been determined recently by Hubbard et al. (1994). In this inactive form, the ATP binding site is blocked by the conformation adopted by the activation loop and Y1162 is engaged in the active site. Insulin activation predisposes the kinase to disengage Y1162, bind ATP and undergo autophosphorylation. Phosphorylation of Y1162 is the key step in IRK activation, whereby a salt bridge is formed with R1131, stabilising the active conformation of the activation loop.

We have used the molecular modelling *Ligand Design* software associated with the *InsightII* program to design inhibitors of kinase activation. This is a method for the design of enzyme inhibitors based on the calculation of interaction sites within the proposed target site of the IRK, followed by alignment and linking of the fragments from a database. We have designed and synthesised various ligands (**1-6**) by condensing 4-hydroxybenzaldehyde with the ethyl esters of glycine, alanine, valine, phenylalanine and GABA, reducing the imines with sodium borohydride, and finally hydrolysing the esters. Our modelling studies show that they bind to the apo form of the receptor at the entrance to the ATP binding site thus preventing access of ATP and autophosphorylation. Our compounds, by binding to this region of the IRK ATP binding site, will have inherent specificity with the residues which line this site and thus overcome the potential for interaction with nucleotide binding sites in other kinases. The inhibitors bind with R1131, D1132, R1136 and Y1162 residues which are instrumental in the autophosphorylation event. D1132 and R1136 are believed to play a role in catalytic proton transfer in the phosphorylation of Y1162 and involvement of these residues in inhibitor binding should prevent the apo form of the IRK from adopting the active form. Compounds **3**, **4** and **5**, with an isopropyl, phenyl or *t*-BOC group respectively, provide interaction with a hydrophobic pocket in the binding site (Schecter et al. 1989). The ethyl ester derivatives have also been prepared which, whilst having a reduced electrostatic contribution to binding, are designed to overcome the problems of cellular penetration which we envisage for the free acids. Calculations were performed employing the AMBER forcefield.

It is envisaged that the data produced by these molecular probes will help in the construction of a comprehensive structure-activity relationship profile to define the parameters required for selective inhibitory activity and gain a greater understanding of the role of IRK autophosphorylation, the role of the kinase activity of the IRK towards exogenous substrates, and the relationship between the two events in triggering the various biochemical pathways in response to insulin signal transduction.



	R_1	R_2	R_3
1	H	COOH	H
2	CH_3	COOH	H
3	$CH(CH_3)_2$	COOH	H
4	CH_2Ph	COOH	H
5	H	$(CH_2)_2COOH$	H
6	H	$(CH_2)_2COOH$	BOC

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