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## Kinase activators as a novel class of antidiabetic agents

Kinases are key regulators in many biochemical pathways, although their direct activation by small molecules is perceived to be less tractable than inhibition, because the regulation of kinases often involves protein–protein interactions, which are difficult to target. These factors have resulted in limited research into the upregulation of kinases as a therapeutic approach compared with kinase inhibition. Some illustrative examples recently described by Simpson, however, have highlighted the potential design principles that might be exploited to discover novel kinase activators [1]. Among them, glucokinase activators (GKAs) appear to be one of the most promising classes for improving current drug treatment of type 2 diabetes mellitus (T2DM) [2].

Glucokinase (GK) plays a key role in whole-body glucose homeostasis by catalyzing the phosphorylation of glucose to glucose-6-phosphate in cells that express this enzyme, such as neuroendocrine cells, hepatocytes, and pancreatic β-cells. GK is the rate-limiting step in glycolysis in liver and β-cells. Considering this central role of GK, there has been significant interest in pharmacological activation of the enzyme and various GKAs have been advanced to clinical studies in T2DM patients. During such clinical studies, however, hypoglycemia has been identified as the key dose limiting adverse effect of GKAs [3].

To overcome the limitations imposed by hypoglycemia risk, several design strategies have emerged in the development of new GKAs. In this context, Pfizer has recently reported the discovery of two novel candidates currently under clinical evaluation in T2DM patients. The development of these discovery programs is based on two different approaches:

(i) Design of 'partial activators' that avoid reducing glucokinase's glucose Km to inappropriately low levels, which led to the discovery of new 2-methylbenzofurans derivatives [4].

(ii) A second strategy based on the hepatoselective tissue distribution to restrict enzyme activation to the liver, leading to the discovery of new imidazo derivatives [5].

As selection criteria, Pfizer defined an *in vitro* screening strategy able to identify activators profiles with reduced hypoglycemic risk. In this cascade, evaluation of glucokinase activation was focused on potent activators (EC50 $< 100$ mM) as well as changes in glucokinase Km (indicated by $\alpha$) and Vmax (indicated by $\beta$) as intrinsic biochemical properties. Attending these parameters, the potential lead compounds can be divided into three categories:

(i) Potential hypoglycemia risk for $\alpha < 0.1$ and $\beta > 1.2$.

(ii) Profile of interest for $\alpha = 0.05$–$0.2$ and $\beta = 0.8$–$1.2$.

(iii) Lack of efficacy risk for $\alpha > 0.1$ and $\beta < 0.8$.

Details in terms of chemistry, SAR and preclinical pharmacokinetics and pharmacodynamics studies, concerning the two families of prototypes will be discussed below.

### Discovery of substituted 2-methylbenzofurans

The 2-methyl benzofuran scaffold (i) was selected as the SAR starting point. Preparation of a variety of structurally diverse analogs was achieved by nucleophilic aromatic substitution using different coupling partners following transamidation. For analogs where the lower aryl/heteroaryl ring was not amenable to installation via an aromatic nucleophilic substitution reaction, palladium- or copper-mediated coupling methods were employed, yielding a diverse family of new compounds which is represented by the general Markush formula (ii).

Analogs were evaluated in the biochemical activation assay. Additionally, human liver microsome stability, passive permeability, kinetic solubility and dofetilide binding, as a surrogate for hERG inhibitory activity, were also assessed. From systematic modifications of the core structure, including replacement of the O-Aromatic substituent with various 6-membered heterocycles and changes of amide substituents, the compound with the optimal balance of potency, activation profile, metabolic stability and solubility was found to be compound (iii).

To test the hypothesis that a glucokinase activator with the profile of (iii) might offer a favorable therapeutic index relative to previous benchmark activators, Pfizer utilized an acute *in vivo* model to conduct a pharmacokinetic/pharmacodynamic assessment of efficacy and hypoglycemia risk. Specifically, an oral glucose tolerance test was conducted in Sprague-Dawley rats. The effect on reducing fasting plasma glucose was determined as a preliminary measure of hypoglycemia safety and demonstrated that the behavior of compound (iii) in this model was consistent with the initial hypothesis that a glucokinase 'partial activator' would have a more beneficial safety profile than full activators.

Based on the promising efficacy and preclinical safety data, (iii) was selected as an early development candidate currently in Phase 1 clinical trials.




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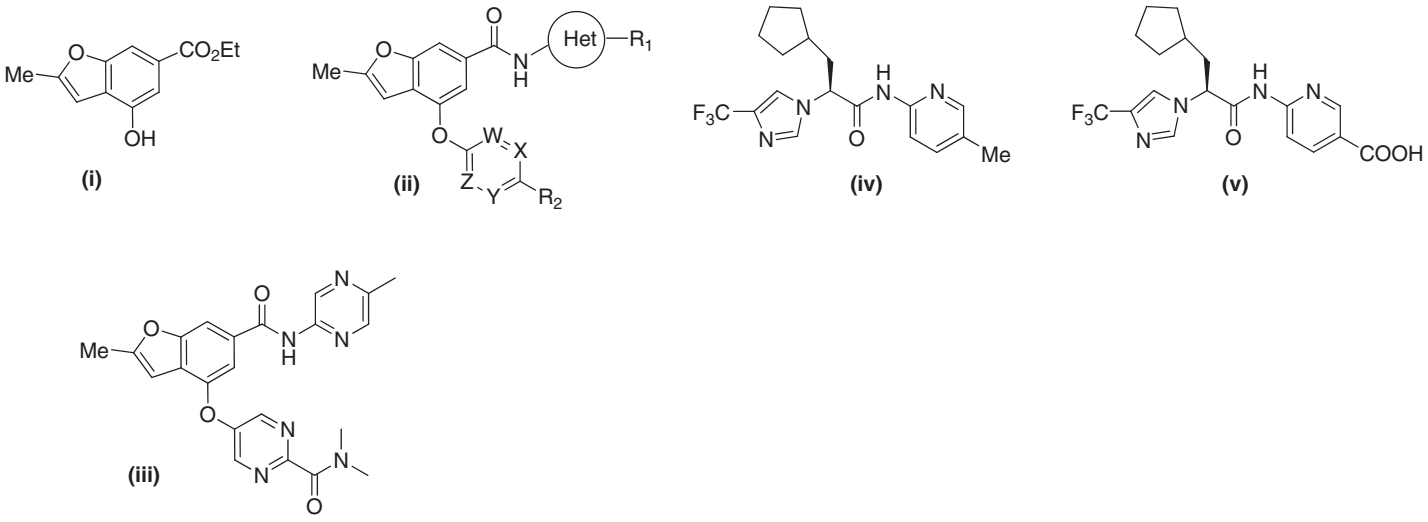


**Discovery of imidazo derivatives**

For the second approach, Pfizer based its strategy in the design of new compounds capable of achieving selective liver activation by incorporating functional groups for recognition and active uptake via liver specific transporters, such as the organic anion transporting polypeptide (OATP).

The starting point for the SAR was the N-heteroarylacetamide (iv) which showed acceptable potency ($EC_{50}$ = 114 nM) and a favorable biochemical activation profile ($\alpha$ = 0.4, $\beta$ = 1.53). In this particular case, molecular modeling studies were particularly useful to the rational design since they showed that the methyl substituent of the 2-aminopyridine was located at a solvent-exposed channel, suggesting that this vector might represent an opportunity to incorporate polar substituents without compromising ligand–protein binding interaction. This evidence, together with the fact that acids are common recognition element of OATP transporters, led the Pfizer team to the replacement of the methyl group by carboxylate (v).

Methyl-heteroarylamide derivatives were synthesized by coupling the corresponding acid chloride with appropriate heteroarylamines. Separately, the synthesis of amides bearing acidic moieties was accomplished using 6-aminonicotinic acid coupled to the corresponding acid chloride.

Pharmacokinetic studies showed values compatible with an OATP substrate and tissue distribution studies revealed a substantial differential between hepatic and pancreatic exposure in both single and repeated oral dose administration in animal models.

To demonstrate the similarities and differences between liver specific and systemic glucokinase activation, the imidazo compound (v) was compared with the previous methylbenzofuran activator (iii). In contrast to the hepatoselective activator (v), (iii) was not a substrate for the OATP transporters and, in rodent tissue distribution studies, was found to be equally distributed between plasma, liver and pancreas.

Whole animal efficacy studies were performed using the Goto–Kakizaki diabetic rat model. The hepatoselective activator (v) had no effect on fasting plasma glucose; however, the systemic activator (iii) reduced glucose levels below euglycemia by the first week of treatment. Finally, as well as the reference systemic activator (iii), compound (v) is currently under clinical evaluation in T2DM patients.

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