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Ligand-based modeling followed by *in vitro* bioassay yielded new potent glucokinase activators

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ABSTRACT

Glucokinase (GK) has received recent interest as a valid antidiabetic target. With this in mind, we applied a computational workflow based on combining pharmacophore modeling and QSAR analysis followed by in silico screening toward the discovery of novel GK activators. Virtual screening identified 10 promising bioactivators from the National Cancer Institute (NCI) list of compounds. The most potent NCI hit illustrated 6.3-fold GK activation at 10 μM. These results demonstrated that our virtual screening protocol was able to identify novel GK activator leads for subsequent development into potential antidiabetic agents.

1. Introduction

Glucokinase (GK), also referred to as hexokinase IV or D, is a member of the hexokinases family. It is predominantly expressed in the liver and pancreas. GK catalyses the phosphorylation of glucose to glucose-6-phosphate (G6P) via adenosine triphosphate (ATP) and Mg^{2+}. Furthermore, GK exerts high control in hepatic glucose metabolism. It acts as key player in the fed state by influencing glucose uptake, while in the fasted state it controls glucose production [1].

Several GK mutations have been linked to abnormalities in blood sugar levels due to either gain or loss of function in GK. Loss-of-function mutations in the GK gene is linked to type 2 diabetes of the young characterized by early onset of mild chronic fasting hyperglycemia [2]. On the other hand, rare activating mutations of GK in man cause hyperinsulinaemia with hypoglycaemia [3].

GK has a unique kinetic profile compared to other hexokinases. It has low affinity to glucose at low glucose concentrations; however, it becomes significantly more active at higher glucose levels. This sigmoidal response to glucose concentration is referred to as 'positive kinetic cooperativity for glucose' and it seems to be related to the unique kinetic transition forms of GK [3].

GK has both open and closed crystal structures in the absence and presence of ligands (glucose and/or GK activators), respectively. It is postulated that in the presence of bound glucose the closed GK conformations are stabilized and GK becomes bioactive (switched on), while the open form is catalytically inactive and is the more stable form in the unbound state (switched off) [4].

The combination of positive kinetic cooperativity, low affinity to glucose at low glucose concentrations, and lack of end-product inhibition render GK activators of excellent potential as treatments of hyperglycemia and diabetes [5]. Activation of GK is expected to lead to better glycemic control through hepatic and pancreatic pathways. Additionally, the reduction in GK activity in response to low glucose levels reduces the possibility of hypoglycaemia during the treatment with GK activators [6].

Initial reports from Hoffmann-LaRoche Inc. about new GK activators (GKAs) (Fig. 1) prompted many pharmaceutical companies to initiate discovery projects to identify small-molecule GKAs as potential treatments for diabetes [5,7]. X-ray crystallographic images of GKAs co-crystallized within GK showed that these compounds bind to an allosteric pocket in the enzyme [3]. GKAs increase

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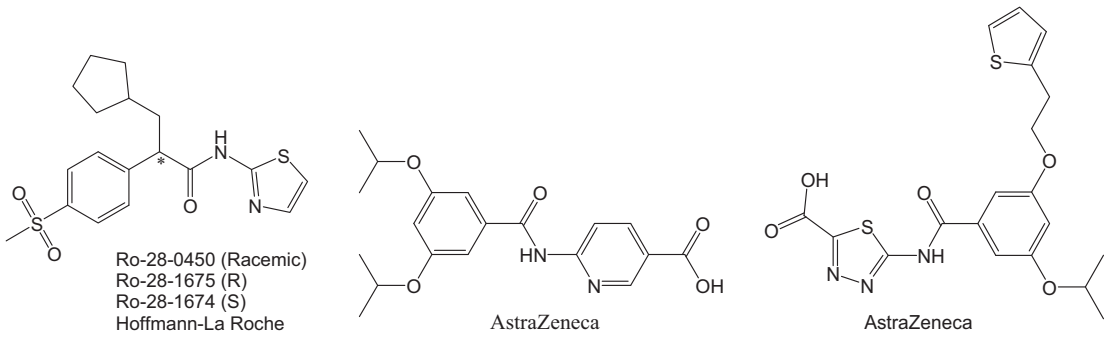

Fig. 1. Selected structures of GKA generated by Hoffmann-LaRoche and AstraZenaca.

the affinity of GK for glucose by stabilizing the closed conformations of this kinase, *i.e.*, in a similar manner to that of GK binding to glucose.

Unsurprisingly, recent discovery and optimization efforts for new GK activators relied heavily on structure-based ligand design [8]. Up to now, 11 X-ray complexes are found in the Protein Data Bank for human GKa (*e.g.*, PDB codes: 3ID8, 3IDH, 3FGU, 3H1V, 3IMX, 3AOI, 3GOI, 3FRO, 3F9M, 1V4S and 1V4T). However, crystallographic structures are restricted by limited resolution [9], crystallization-related artifacts of the ligand–protein complex [10] and negligence of protein anisotropic motion and conformational substrates [11].

The continuous interest in designing new GK activators, combined with problems of crystallographic structures and the induced-fit flexibility documented for GK [3,7,12] encouraged us to investigate the prospects of producing ligand-based pharmacophore(s) incorporated within quantitative structure–activity (QSAR) equation. This combination is independent of the structure of the binding site and thus should avoid the downsides of structure-based methodologies; furthermore, the resulting pharmacophore(s) can be used as search query(ies) for exploration of new GK activators.

We previously reported the use of this interesting methodology toward the discovery of new leads for glycogen synthase kinase 3β [13], hormone sensitive lipase [14], bacterial MurF [15], protein tyrosine phosphatase1B [16], influenza neuraminidase [17], β-secretase [18], CDK1 [19], cholesteryl ester transfer protein [20], and β-D-galactosidase [21].

Our computational workflow started by generating many reasonable pharmacophores for a list GK activators using CATALYST-HYPOGEN [22]. Subsequently, genetic algorithm (GFA) coupled with multiple linear regression (MLR) were implemented to search for optimal quantitative structure–activity relationship (QSAR) that combine high-quality binding pharmacophore with other molecular descriptors that can explain bioactivity variation across the collected list of GK activators. The QSAR-selected pharmacophore was validated using receiver operating characteristic (ROC) curve analysis, and was subsequently employed to mine the national cancer institute's (NCI) compound database for new GK activators. Captured hits were evaluated *in vitro*.

2. Materials and methods

2.1. Molecular modeling

2.1.1. Software and hardware

Pharmacophore and QSAR modeling studies were performed using CATALYST (HYPOGEN module, version 4.11, from Accelrys, USA), CERIUS2 (version 4.10, from Accelrys, USA) and Discovery Studio (version 2.5.5, from Accelrys, USA) software suites. The chemical structures were drawn using ChemDraw Ultra 7.0 (Cambridge Soft Corp., USA).

2.1.2. Data set and conformational analysis

The structures of 30 GK activators (Table 1) were collected from the literature [23a,23b]. Their *in vitro* bioactivities were expressed as concentrations that activated GK by 50% (EC_{50}). Table 1 shows the collected structures and their corresponding EC_{50} values. The logarithm of EC_{50} (μM) values were used in modeling to correlate data linearly to the free energy change. However, in cases where EC_{50} values were expressed as being >10 μM (*e.g.*, **2–6**, **8** and **10**) they were assumed to be 200 μM to maintain 4 log cycles difference from the most potent compound (**28**, EC_{50} = 0.02 μM). This bioactivity spread is essential requirement for CATALYST pharmacophore modeling [22,24]. The logarithmic transformation of EC_{50} values is expected to reduce any possible errors resulting from this supposition.

The chemical structures of the activators were drawn and saved as mol files. Then, they were converted into corresponding 3D structures and minimized to the closest local energy minima using the CHARMm force field within CATALYST. The resulting 3D conformers were utilized as starting points for conformational analysis.

The conformational surface of each activator (**1–30**, Table 1) was explored using the CHARMm force field implemented within CATALYST via the "best conformer generation" option. Conformational ensembles were generated for each training compound with energy threshold of 20 kcal/mol from the closest local minimum with a maximum limit of 250 conformers per molecule. The conformation search procedure implements a "poling algorithm" that penalizes closely related conformers to avoid entrapment in certain local minimum during conformational sampling [22], which endanger pharmacophore generation and subsequent in silico screening [25].

2.1.3. Pharmacophoric hypotheses generation

The training compounds (30 molecules) together with their associated conformational models were listed into a single spreadsheet with their EC_{50} values combined with an "Uncertainty" of 3. This value assumes that the actual EC_{50} value of any activator is situated somewhere in an interval ranging from one-third to three-times the reported EC_{50} value [24b–d].

A structurally diverse training subset (Table 2) was selected for pharmacophore exploration through four modeling runs, as in Table 3. Different pharmacophores were produced by changing the interfeature spacing and the count of permissible features in the resulting models (Table 3). Section SM-1 under Supplementary Materials describes how CATALYST-HYPOGEN generates pharmacophoric models [24b–d]. Ultimately, our exploration efforts (4 automatic runs, Tables 2 and 3) yielded 40 binding models of variable qualities.