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Pharmacophore modeling, virtual screening, molecular docking studies and density functional theory approaches to identify novel ketohexokinase (KHK) inhibitors

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ABSTRACT

Fructose catabolism starts with phosphorylation of D-fructose to fructose 1-phosphate, which is performed by ketohexokinase (KHK). Fructose metabolism may be the key to understand the long-term consumption of fructose in human's obesity, diabetes and metabolic states in western populations. The inhibition of KHK has medicinally potential roles in fructose metabolism and the metabolic syndrome. To identify the essential chemical features for KHK inhibition, a three-dimensional (3D) chemical-feature-based QSAR pharmacophore model was developed for the first time by using Discovery Studio v2.5 (DS). The best pharmacophore hypothesis (Hypo1) consisting two hydrogen bond donor, two hydrophobic features and has exhibited high correlation co-efficient (0.97), cost difference (76.1) and low RMS (0.66) value. The robustness and predictability of Hypo1 was validated by fisher's randomization method, test set, and the decoy set. Subsequently, chemical databases like NCI, Chembridge and Maybridge were screened for validated Hypo1. The screened compounds were further analyzed by applying drug-like filters such as Lipinski's rule of five, ADME properties, and molecular docking studies. Further, the highest occupied molecular orbital, lowest unoccupied molecular orbital and energy gap values were calculated for the hits compounds using density functional theory. Finally, 3 hit compounds were selected based on their good molecular interactions with key amino acids in the KHK active site, GOLD fitness score, and lowest energy gaps.

1. Introduction

Fructose is a kind of monosaccharide and it is present in honey, fruits and vegetables. Fructose is transported into the liver with the help of GLUT5 and/or GLUT2 transporters. The ketohexokinase (KHK) is the first enzyme in the degradation of fructose and it is phosphorylated as fructose 1-phosphate by KHK in the liver and in kidney (Arooj et al., 2013; Cirillo et al., 2009). When fructose is phosphorylated by KHK, adenosine triphosphate (ATP) is consumed with the formation of adenosine monophosphate (AMP). The high level of KHK was also found in the renal cortex, small intestine, and pancreas (Kasim-Karakas et al., 1996; Koo et al., 2008). The elevated fructose ingestion promotes various metabolic disturbances in animal models, including weight gain, hyperlipidemia, hypertension, and insulin resistance (Hwang et al., 1987; Kasim-Karakas et al., 1996; Martinez et al., 1994; Reiser and Hallfrisch, 1977; Zavaroni et al., 1980). The consumption of fructose in humans increases energy intake, fat mass, body weight, blood pressure, and plasma triglycerides (Raben et al., 2002; Teff et al., 2004).

The metabolism of fructose results in brain exerts an orexigenic effect. Due to fructose metabolism the level of Malonyl-CoA

Abbreviations: KHK, ketohexokinase; DS, Discovery Studio v2.5; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; HY, hydrophobic; RMS, root mean square; EF, enrichment factor; GH, goodness of hit; ADME, absorption, distribution, metabolism, and excretion; BBB, blood–brain barrier; ADT, AutoDock tool; DFT, density functional theory; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital.

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decreases in the hypothalamus, which results in increased food intake and obesity. In contrast the metabolism of glucose in hypothalamus results is an increased level of Malonyl COA which is correlated with the reduced expression of several orexigenic (appetite stimulant) peptides concomitant with the activated of several anorexiginic (appetite depressed) peptides. These changes in neuropeptides expression result in suppressed food intake and increased energy expenditure.

The excessive fructose absorption stimulates de novo lipogenesis via up-regulation of gene expression, favors re-esterification (Kok et al., 1996; Miyazaki et al., 2004) of fatty acids, and increases the production of very low-density lipoprotein (VLDL) (Mayes, 1993) particles. The chronic high-fructose diets elevate free fatty acids and triglycerides, which impair glucose usage in muscle tissue and increase the rate of lipolysis in adipose tissue (Mayes, 1993). The elevated triglycerides could impede insulin-signaling pathways (Dresner et al., 1999; Koteish and Diehl, 2001; Ueno et al., 2000), support chronic inflammation (Holven et al., 2006; Oron-Herman et al., 2003; Wu et al., 2004), and lead to glyco lipid toxicity (Swanson et al., 1992) with the possible failure of pancreatic β-cells (Lewis et al., 2002).

The hepatic fructokinase deficiency is caused by recessive mutations in KHK gene. This is characterized by a large and insistent increase in blood fructose after the ingestion of fructose, sucrose or sorbitol and the excretion of 10–20% of the consumed load in the urine (Bonthron et al., 1994; Cox, 2001). The removal of fructose in the urine is not constant; it depends largely on dietary intake. The substitutions of amino acids results in the mutation of Gly40Arg and Ala43Thr, which caused disease and affect the individuals and family (Bonthron et al., 1994).

The KHK belongs to the family of prokaryotic ribokinases and furanose sugar kinases. Some other furanose sugar can also act as KHK substrate. Two KHK protein isoforms are encrypted by alternatively intertwined KHK mRNAs. These were stated as KHK-C (mainly central; hepatic/renal/intestinal) and KHK-A (more extensively distributed but with a low manifestation level) (Hayward and Bonthron, 1998) also physiological function is not currently defined for KHK-A. Using recombinant proteins, several classes of reports suggest both KHK isoforms were enzymatically energetic; however, KHK-A is significantly more thermostable and had a lesser affinity for fructose than KHK-C (Asipu et al., 2003). Thus, KHK inhibitors provide a pharmacological tool and topical applications might offer a potential treatment against various metabolic syndromes and inborn error of metabolism. According to our understanding, till now no compound had arrived at the clinical trials, justified the newness of KHK agonists. Therefore, inhibitors of KHK were very eye-catching to the pharmaceutical industry and researchers.

A pharmacophore model was a group of steric and electronic features that is necessary to assure the best supramolecular interactions with an exact biological target and to activate or block its biological reaction (Yang, 2010). The pharmacophore models were generally categorized as the ligand-based method and structure based methods. In KHK we approach ligand-based pharmacophore modeling had become a main computational strategy for facilitating drug discovery in the lack of a macromolecular target structure (Niu et al., 2014; Yang, 2010). It was accomplished by extracting mutual chemical features from 3D structures of a known ligands characteristic of important interactions between the ligands and a specific macromolecular target.

In the present study, 3D QSAR pharmacophore model for KHK inhibitors have developed using Discovery Studio v2.5 (DS). The generated pharmacophore hypothesis was validated by fisher's randomization, test set, and decoy set methods. The best pharmacophore hypothesis was used as the 3D query to search various chemical databases (Heikamp and Bajorath, 2013). The hit compounds were subjected to filter by and ADME properties and Lipinski's rule of five. The molecular docking studies were done with the hit compounds to find the binding affinity between ligand and critical amino acids present in the active site of the receptor. The Orbital energy values were also calculated to find out the reactivity of the lead compounds by using density functional theory (DFT).

2. Materials and methods

2.1. Dataset preparation

We have selected 52 structurally diverse compounds with the reported inhibitory activity values from the literature (Maryanoff et al., 2012; Zhang et al., 2011). To attain the best hypothesis, the training set obeys the 3D quantitative structure-activity relationship generation. The training set should contain 16 compounds with good structural diverse, covering an overall activity range of 4 orders of magnitude. The best active compounds should inexorably be encompassed in the training set and all biologically relevant data would be achieved by homogeneous processes and expressed as IC_{50} (i.e., concentration of compound required to inhibit 50% of KHK). All the structures were minimized using smart minimizer algorithm which makes 1000 steps of steepest decent with a root mean square gradient of 0.1, followed by conjugated gradient minimization. Amongst 52 molecules, 21 compounds were selected as a training set (Fig. 1) and the remaining 31 compounds were assumed as the test set (Fig. S1). The training set was chosen based on the structural diversity and extensive coverage of the activity values which spans transversely a wide range from 0.10 nM to 10,000 nM. Further, the compounds are separated into three categories: highly active compounds ($IC_{50} < 100$ nM), moderately active compounds ($100\text{ nM} \leq IC_{50} < 1000$ nM) and inactive compounds ($IC_{50} \geq 1000$ nM). The geometry of all compounds was built and energy minimization process was achieved using CHARMM force field parameter (Brooks et al., 1983). Poling algorithm was applied to generate a maximum of 255 diverse conformations with the energy threshold of 20 kcal/mol (Smellie et al., 1995).

2.2. Pharmacophore generation

In the pharmacophore model generation, the primary pharmacophore features, hydrogen-bond donor, hydrogen-bond acceptor, and hydrophobic moiety were specified based on the common features pharmacophore. While generating pharmacophore model, a minimum of 0 and maximum count of 5 for all the features were selected and used to form a series of hypotheses using an uncertainty value of 2. *HypoGen* generates quantitative pharmacophore models based on the most active compounds from the training set. In addition, the activity of each training set compounds were estimated using the regression parameters and computed by the regression analysis using the relationship of experimental and predicted activities. The top 10 hypotheses with significant statistical parameters were generated on the basis of a highest correlation coefficient (r), low RMS and cost function.

2.3. Pharmacophore validation

The best pharmacophore model was validated by three different methods: Fischer randomization method, test set prediction, and decoy test. In the first method, the statistical validation was carried out by Fischer's randomization method to assess the significance of hypothesis generated by HypoGen. The desired confidence level

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