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Research Article

Structure based design, synthesis and biological evaluation of amino phosphonate derivatives as human glucokinase activators

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ABSTRACT

Glucokinase (GK) is a potential therapeutic target of type 2 diabetes and GK activators (GKAs) represent a promising class of small organic molecules which enhance GK activity. Based on the configuration and conformation of the allosteric site of GK, we have designed a novel class of amino phosphonate derivatives in order to develop potent GKAs. The QSAR model developed using numerous descriptors revealed its potential with the best effective statistical values of RMSE = 1.52 and r^2 = 0.30. Moreover, application of this model on the present test set GKAs proved to be worthy to predict their activities as a better linear relationship was observed with RMSE = 0.14 and r^2 = 0.88. ADME studies and Lipinski filters encouraged them as safer therapeutics. The molecular dynamics and docking studies against the GK allosteric site revealed that all GKAs bind with best affinities and the complexes are strengthened by H-bonding, phosphonate salt bridges, hydrophobic and arene cat ionic interactions. Finally, in *vitro* evaluation with human liver GK revealed their potential to increase the GK activity by different folds. The results from QSAR, ADME, molecular docking and *in vitro* assays strongly suggested that the present molecules could be used as effective and safer therapeutics to control and manage type 2 diabetes.

1. Introduction

Type 2 diabetes mellitus is a progressive heterogeneous disease affecting millions of people every year throughout the world (Hu, 2011). For the first time in 1990, a search began for molecules that can activate the pivotal enzyme Glucokinase (GK) and maintains glucose homeostasis (Cuesta-Munoz et al., 2001; Grimsby et al., 2001; Matschinsky et al., 2006). GK is a potential diabetes gene where the minute decrease in its activity will result in a diabetic condition which is also linked with the mutations in its coding region (Hattersley et al., 1992; Froguel et al., 1992). Development of GK activators (GKAs) converged in the exciting new concept that, this glycolytic enzyme is endowed with an allosteric activator site that is switched on by activator molecules (Coghlan and Leighton, 2008; Guertin and Grimsby, 2006) and hence became a strong candidate drug target (Matschinsky et al., 2006; Coghlan and Leighton, 2008; Agius, 2007; Liu et al., 2012). GKAs bind with allosteric site and enhance the GK activity through conformational transitions mediated by local Y214 residue (Grimsby et al., 2003; Brocklehurst et al., 2004; Kamata et al., 2004; Hinklin et al., 2013).

Structure based drug discovery process became a most significant popular tool and in the present study it has been given a major importance to design and develop a novel class of GKAs (DePristo et al., 2004; Klebe, 2006; Steuber et al., 2006; Mallipeddi et al., 2014; Song et al., 2009; Parrill, 1997). The availability of plenty of experimental information about GK structure provided a strong basis and support, especially with the detailed information about the catalytic and allosteric sites. We have focused on the

Abbreviations: GK, Glucokinase; GKA, glucokinase activators; QSAR, Quantitative Structure Activity Relationship; RMSE, root mean square error; RMSD, root mean square devaition; ADME, absorption, distribution, metabolism, and excretion; IR, infra red; NMR, nuclear magnetic resonance; LC, liquid chromatography; MSA, methane sulphonic acid; MOE, molecular operating environment; PCA, principal components analysis; Caco-2, colon adenocarcinoma; MDCK, Madin Darby Canine kidney; BBB, blood brain barrier; HIA, human intestinal absorption; PDB, protein data bank; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; NADPH, nicotinamide adenine dinucleotide phosphate; NADP, nicotinamide adenine dinucleotide phosphate; DMSO, Di Methyl Sulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ATP, adenosine triphosphate; EC, effective concentration.

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virtual fitting of novel leads into the GK allosteric site with complementary orietnations. We employed the algorithms that rely on the scoring functions and force fields to calculate the affinity interactions in terms of docking energies between the complimentary groups and/or functional groups of leads with the R-groups of amino acid residues of the allosteric site (Menikarachchi and Gascon, 2010). The attractive/repulsive interactions among them decide the orientation and behaviour of the lead in the binding site and even suggest more modifications to enhance or modify the reactivity of molecules. By virtue of these considerations, we designed some potential leads that could interact with the allosteric site of GK to enhance its activity.

The GK allosteric site is composed of specifically two kinds of residues i.e. polar and hydrophobic residues. The polar residues Y61, R63, S64, T65, Y214, Y215, C220 and M235 contain the R-groups such as —OH, —SH and —NH2 where the polarity of these groups results in the formation of hydrogen bonds with other polar groups of ligands through partial charge attraction. The hydrophobic residues such as V62, I211, L451, V452 and V455 contain the R-groups without electro negative atoms and they can form the hydrophobic interactions with small molecules (Fig. 1). This preliminary information about the R-groups of such amino acids suggested the complementary functional groups in the leads so that efficient interactions can be made accordingly.

We have chosen amino, phosphate and aromatic ring structures as starting fragments and joined them in different orientations to develop the novel potent leads that can specifically interact with the residues of allosteric site. The amino group has the ability to form hydrogen bonds with the polar residues, whereas the phosphate moiety is hydrophilic in nature and thus interact with the polar residues by means of hydrogen bonding. The added advantage of phosphate moiety is, it is rich in electro negative atoms and more over it could enhance the reactivity of the molecule. As the allosteric site is having multiple tyrosine residues which are aromatic and especially Y215 residue involved in the allosteric regulations, aromatic structures were used to build the leads where they may form more stable π-π stacking interactions. Based on these analyses our efforts came out with the design of a novel class of amino phosphonate molecules. The pharmacological and drug like properties were studied by QSAR, Lipinski and ADME approaches. This study provided a clear insight into how these molecules achieved their pharmacological effects through *in silico* followed by *in vitro* means and may further contribute to our understanding and design of small molecule GKAs as potential therapeutics for the treatment of diabetes.

2. Materials and methods

Chemicals were obtained from Sigma–Aldrich, used as such without further purification. All solvents (AR or extra pure grade) used for spectroscopic and other physical studies were further purified by literature methods. All operations were performed under nitrogen atmosphere using standard glass wares. Melting points were determined using a calibrated thermometer by Guna Digital Melting Point apparatus. Elemental analyses were performed from University of Hyderabad, Hyderabad. IR Spectra were recorded in Environmental Engineering Laboratory, Sri Venkateswara University, Tirupati as KBr discs on a Nicolet 380 FT-IR spectrophotometer. ^{13}C NMR spectra were recorded as solutions in DMSO-d_6 on a Bruker AMX 400 MHz spectrometer operating at 100 MHz for ^{13}C and 161.9 MHz for ^{31}P. The ^{1}H and ^{13}C chemical shifts were referenced to tetramethylsilane and ^{31}P chemical shifts to 85% H_3PO_4. LC mass spectra were recorded on a Jeol SX 102 DA/600 Mass Spectrometer.

2.1. Synthesis of GKAs

To a stirred solution of diethyl phosphite, ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylate and substituted amines were added in the presence of a catalyst under solvent free conditions to form new α-amino phosphonates in high yields (75–96%). All the substituted amines were readily reacted with diethyl phosphite and ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylate under similar reaction conditions to afford the corresponding α-amino phosphonates. In all the cases, the reactions proceeded smoothly in refluxed temperature. The reactions were clean and completed within 5–6 h. The reaction conditions were very mild and the α-amino phosphonates were

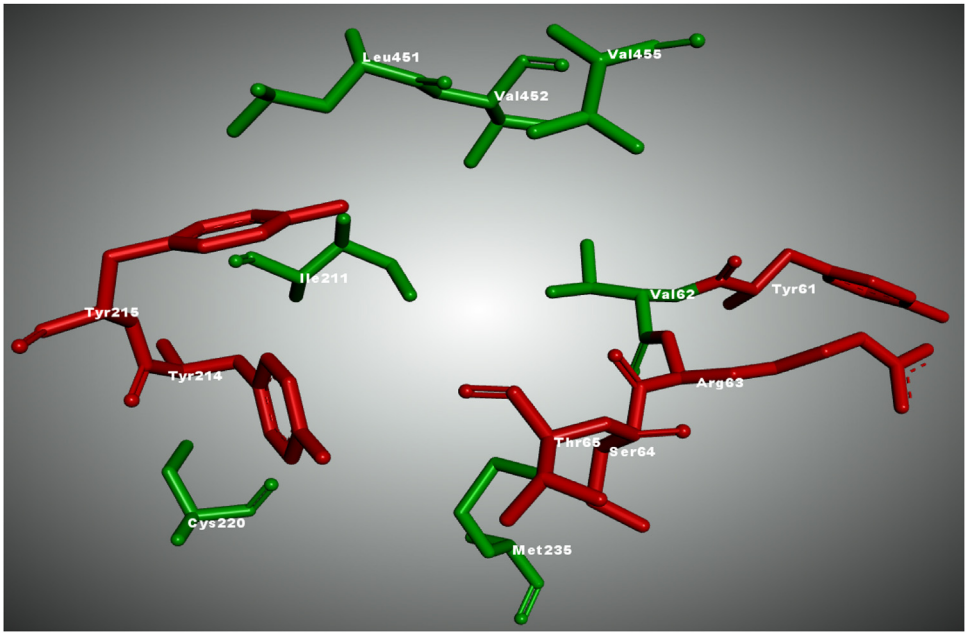

Fig. 1. Allosteric site residues of GK. Hydrophobic residues are shown in green color and polar residues in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)