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Original Article

DISCOVERY OF NOVEL IKK-B INHIBITOR BY STRUCTURAL MODIFICATIONS OF CHLORPROPAMIDE AND NATEGLINIDE

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ABSTRACT

Objective: Diabetes Mellitus (Type II) is one of the predominant diseases which has seen rise globally. Kappa-B kinase beta (IKK-β) - a protein subunit of IKappa-B kinase, is an emerging target for treatment of non-insulin dependent diabetes mellitus (NIDDM). Chlorpropamide(diabinese) and nateglinide (starlix) are two anti-diabetic, FDA approved drugs but shows onset of drug resistance at later stages of therapy. In this study, an *in-silico* approach has been attempted to modify these ligands for inhibition of IKK-β protein.

Methods: The structure of IKK-β has been determined through homology modelling. Stereochemical properties of structure were checked to validate stability of modelled structure. Ligand-protein interaction was studied through molecular docking to find out lead for IKK-β inhibition using Molecular Docking server. Drug safety of studied drugs was determined using OSIRIS.

Results: Best drug has been reported on the basis of its interaction with IKK-β, its inhibitory property against IKK-β and drug safety.

Conclusion: The work provides insight for molecular understanding of IKK-β and can be used for development of anti-diabetic drugs.

Keywords: Diabinese, Starlix, OSIRIS, Homology Modelling, Molecular Docking.

INTRODUCTION

Diabetes mellitus is a global health epidemic, affecting approximately 171 million people in 2000 and WHO projects that diabetes deaths will double between 2005 and 2030 [1]. Approximately 90% of people with diabetes have diabetes mellitus [2]. The cause of such a tremendous increase ranges from obesity, sedentary lifestyle, to family history or autoimmunity [3, 4]. Diabetes leads to complications associated with other diseases such as myocardial infarction, nephropathy, retinopathy and neuropathy which emphasises on the need to diagnose it at an early stage and the requirement of developing its effective treatment [5-7].

In cytoplasm, phosphorylation of inhibitory protein IKappa B (IKB) is mediated by kinase complex known as IKK [8]. IKB Kinase (IKK) complex consists of 3 subunits: α, β and γ. IKK-α, IKK-β together are catalytic sub-units and IKK-γ is the regulatory unit [9]. IKK activation (phosphorylation) causes release of the subunit IKK-β which further goes and phosphorylates IRS-1 (Fig. 1) [10]. IKK-β plays a significant role in counteracting action of insulin by directly phosphorylating IRS-1 on serine residues [11]. Thus inhibition of IKK-β can be a viable therapeutic avenue for diabetes [12].

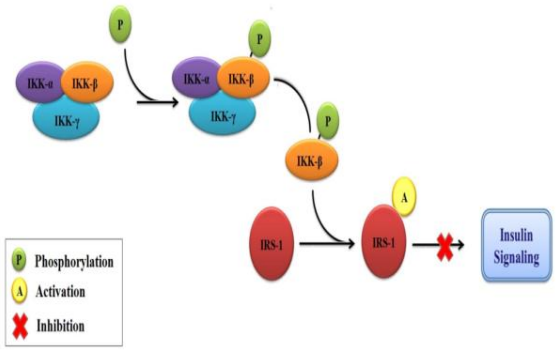

Fig. 1: Insulin signalling inhibition pathway through IKK- β phosphorylation.

Several classes of anti-diabetic oral drugs are available in market, but emergence of drug resistance because of their prolonged use leads to demand for discovery of new drugs [12, 13]. Chlorpropamide is an oral anti-hyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin [13]. Nateglinide is an oral anti-hyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the meglitinide class of short-acting insulin secretagogues, which act by binding to β cells of the pancreas to stimulate insulin release (Fig. 2) [14, 15]. New ligands can be obtained by simple modification of the existing drugs for NIDDM - chlorpropamide and nateglinide [16, 17].

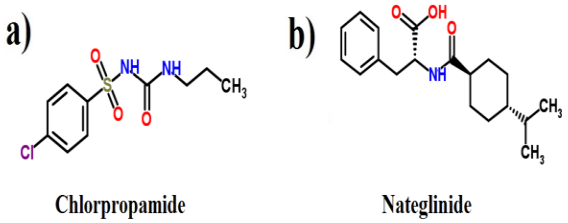

Fig. 2: Two dimensional structure (a) Chlorpropamide (b) Nateglinide.

This work is an *in-silico* approach to study inhibition of the IKK-β phosphorylation by the modified ligands. Due to the difficulties and economic cost of the experimental methods for determining the structures of complexes, computational methods such as molecular docking are desired for predicting putative binding modes and affinities. For the drugs having low inhibition, functional modification by substitution is done to make the drug more feasible and efficient in binding to IKK-β. The work will help in molecular understanding of IKK-β inhibition and identification of new lead structure for development of drug against NIDDM.

MATERIALS AND METHODS

Homology modelling

The sequence of IKK-β was retrieved from NCBI (Accession ID O14920). To find out whether the structure of target protein was available, blastp was performed against structures present in Protein Data Bank. It was checked that none of the BLAST hits is totally identical to our target protein. Homology modelling is the most accurate computational method for translating an amino acid sequence into a protein structure [18]. For building the structure of IKK-β, homology modelling of target protein was performed using Modeller 9.12 [19]. Template structure (PDB ID- 4KIK chain B) with 88% sequence coverage and 99% identity was selected for homology modelling of IKK-β (Fig.3). The stability of IKK-β structure thus obtained was further verified using Ramachandran Plot generated by PROCHECK server [20].

Fig. 3: Sequence alignment of target sequence (Query) and homology modelling template identified using Swiss Model (Sbjct).

Energy minimization

To obtain the minimum energy conformation of the modelled structure, its energy minimization was performed using VegaZZ [21]. The protein structure was minimized using steepest descent algorithm and CHARMM forcefield. Superim position of energy minimised structure with respect to the model structure, was performed using Discovery studio visualizer [22]. RMSD value was calculated to study the deviation of energy stabilized structure from the modelled structure.

Ligand modification

As mentioned earlier, derivatives of chlorpropamide and nateglinide were selected for study of its interaction with IKK-β. The SMILES string of the parent ligands were taken from Drug Bank and submitted in CORINA server [23] to generate their 3D structures of chlorpropamide and nateglinide. In an attempt to obtain optimum ligands, modifications by substituting few hydrogens with other functional groups was done using VEGAZZ (Fig. 4).All ligand structures were subjected to energy minimization through VEGAZZ using the same parameters as mentioned for protein structure energy minimization.

Ligand-protein interaction

Since Kuntz and colleagues' pioneering work, significant progress has been made in docking research to improve the computational speed and accuracy [15]. Among them, protein-ligand docking is a particularly essential because of its importance in structure-based drug design. Molecular Docking Server is commercially available web server, which facilitates ligand-protein docking and analysis. It performs docking of the ligand on the target, by automatically generating the best sites possible for their binding [24]. The energy minimized ligands were docked to study the binding patterns with the target protein IKK-β. Free binding energy and interaction sites of each IKK-β-ligand complexes were analysed to study their stability.

Toxicity risk assessment

Properties of ligands were checked using Osiris Property Explorer [25] to ensure the safety of ligands. It was checked if the ligands are mutagenic, tumorigenic, irritant or possesses any adverse effect in reproductive system based on which the drug score of each was calculated.

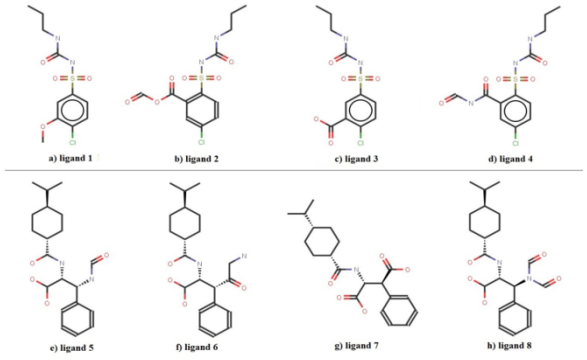

Fig. 4: Modified ligands : (a) ligand 1:1-(4-chloro-3-methoxybenzenesulfonyl)-3-propylurea, (b) ligand 2: formyl 5-chloro-2-{[propylcarbamoyl)amino]sulfonyl}benzoate,(c) ligand 3:2-chloro-5-{[(propylcarbamoyl)amino]sulfonyl}benzoicacid, (d) ligand 4: N-(5-chloro-2-{[propylcarbamoyl)amino]sulfonyl}benzoyl)formamide, (e) ligand 5: N-[(1R,2R)-3,3–dihydroxy-2-({hydroxy[(1r,4r)-4-(propan-2-yl)cyclohexyl]methyl}amino)-1-phenylpropyl]formamide, (f) ligand 6: (3R,4R)-1-amino-5,5-dihydroxy-4-({hydroxy[(1r,4r)-4-(propan-2-yl)cyclohexyl]methyl}amino)-3-phenylpentan-2-one, (g) ligand 7: (2S,3R)-2-phenyl-3-{[(1s,4s)-4-(propan-2-yl)cyclohexyl]formamido}butanedioicacid, (h) ligand 8: N-(1R,2R)-3,3-dihydroxy-2-({hydroxy[(1r,4r)-4-(propan-2-yl)cyclohexylmethyl}amino)-1-phenylpropyl]-N-formylformamide.

RESULTS AND DISCUSSION

Homology modelling and structure validation

As the crystallographic structure of IKK-β has not been determined yet, *in-silico* model of IKK-β was generated for understanding its ligand interaction mechanism.

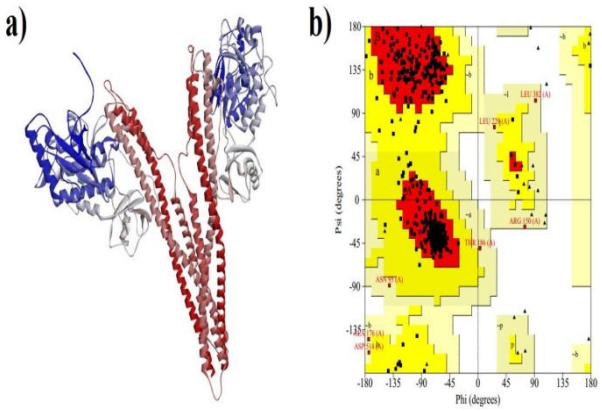

Fig. 5: IKK-β model structure and validation (a) Structure of IKK-β model viewed using- Discovery studio 3.5 visualizer. (b) Ramachandran plot of amino acid residues of IKK-β generated using PROCHECK server. 92.2% residues are in most favoured region, 6.6% residues are in additional allowed region, 1.2% residues are in generously allowed region and 0.0% residues are in disallowed region of Ramachandran plot.

The structure having similar sequence will exhibit similar structure forms the basis of homology modelling. Availability of template structure for most of the residues made the generation of structure

model possible, with high precision and accuracy. The 3D structure of IKK-β as obtained by homology modelling using modeller 9.12 is shown in Fig. 5a. In stereo chemical study of structure, 92.2% residues were found in most favoured region, 6.6% in additional allowed region, 1.2% in generously allowed region and no residues in disallowed region of Ramachandran Plot (Fig. 5b). IKK-β is stereo chemically favoured, suggesting that the modelled structure is of good quality [20].

In VEGAZZ, when IKK-β was subjected to energy minimization, it reached its minimum energy conformation in 3000 steps. The energy of IKK-β structure before and after energy minimization was found to be -565287.7 kJ/mol and -806088.0 kJ/mol respectively, using YASARA program. In Discovery studio visualizer 3.5 [22], energy minimized structure superimposed to C-alpha of the original model structure (Fig. 6) showed RMSD of 0.43 Å. As the deviation in structure backbone is very low, it implies that structure model is having a stable conformation.

Ligand-protein interaction

The stability of protein complex can be measured through its binding energy. The complexes with lower binding energy are more stable and thermodynamically favoured. The binding free energies (kcal/mol)of each protein ligand complex obtained by Molecular Docking Sever are summarized in Table 1.

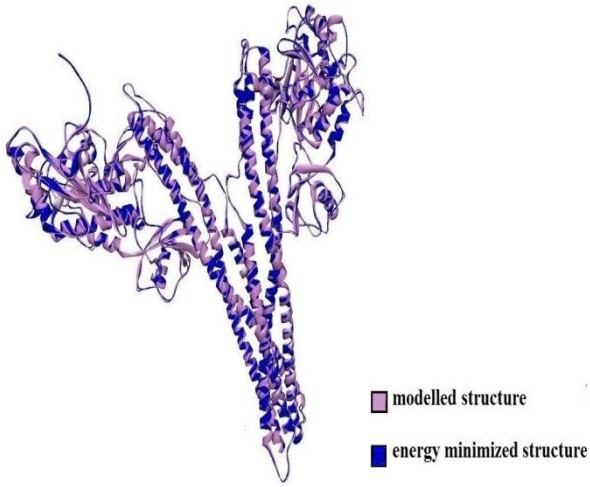

Fig. 6: Superimposition of model structure with respect to energy minimized structure as viewed in Discovery studio 3.5 Visualizer.

Table 1: Docking results and drug safety

S. No.	Ligand	Binding energy (kcal/mol)	Toxicity risk	Drug score
1	Ligand 1	-4.51	No	0.87
2	Ligand 2	-4.11	Yes	0.17
3	Ligand 3	-3.99	No	0.50
4	Ligand 4	-4.23	No	0.84
5	Ligand 5	-4.30	No	0.42
6	Ligand 6	-3.70	No	0.41
7	Ligand 7	-4.21	No	0.41
8	Ligand 8	-3.27	No	0.38

All protein-ligand complexes are having low binding energy, suggesting that they are stable. Chlorpropamide derived molecule Ligand1 forms most stable complex with IKK-β, having binding energy -4.51 kcal/mol. Chlorpropamide derivative - ligand1possess the highest drug score. Toxicity risk study reveals that ligand 2 may be mutagenic, tumorigenic or irritant. Ligands other than ligand2 were found to be safe (Table 1). Based on cumulative analysis of binding energy, toxicity risk and drug score, ligand1 [1-(4-chloro-3-methoxybenzenesulfonyl) -3-propylurea] can be accepted as potential inhibitor of IKK β.

Ligand1 forms hydrogen bond interaction with ARG124, ASN383, GLY385, THR387, ASP389, MET390, and GLN455 (Fig. 7). The graphical representation of hydrogen bonds formed by ligand1 and IKK-β is shown in Fig. 8. Abundance in number of interactions between drug and target shows that protein ligand complex is stable.

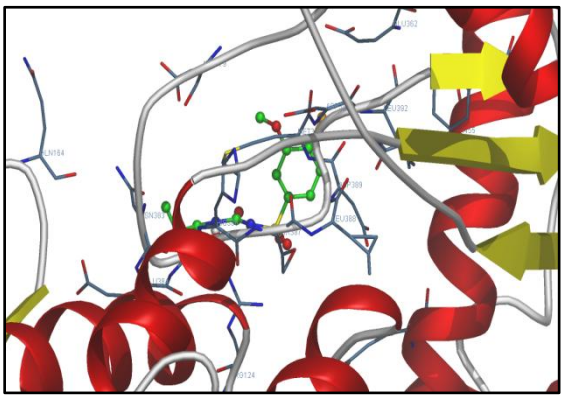

Fig. 7: Interaction of ligand 1 with IKK-β viewed using Molecular Docking Server.

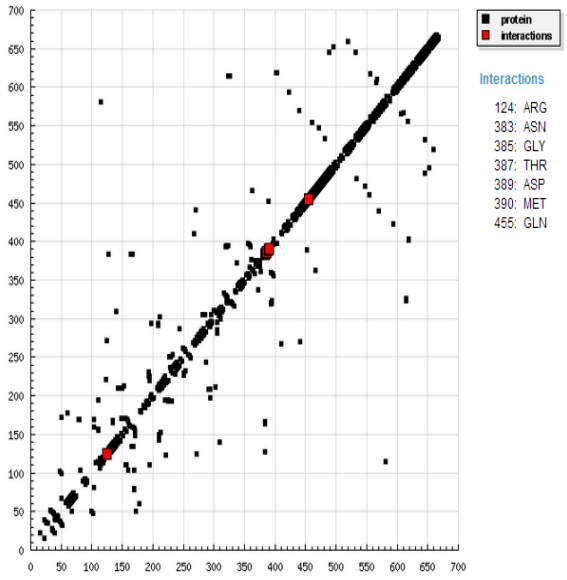

Fig. 8: Graphical representation of hydrogen bonds in IKK-β-ligand1 complex. Red spots indicate the position of hydrogen bonds formed between ligand 1 and IKK-β.

Drug safety

Drug safety study of Ligand1 done by OSIRIS Property Explorer is shown in Fig.9. Ligand1[1-(4-chloro-3-methoxybenzenesulfonyl)-3-propylurea] was not found to violate any of toxicity tests and have high drug likeliness and drug score.

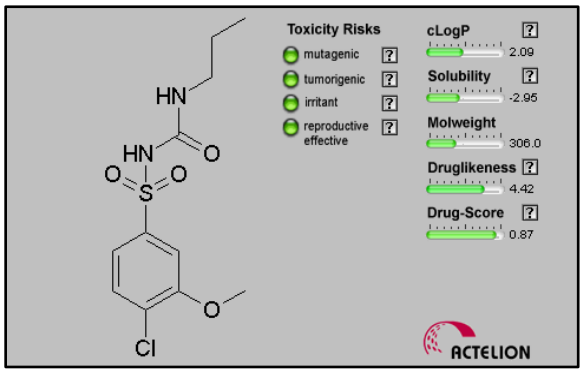

Fig. 9: Properties of ligand 1 calculated using OSIRIS Property Explorer.

CONCLUSION

IKK-β is a potential drug target for NIDDM. Due to emerging drug resistance there is demand for discovery of new drug. As crystallographic structure of IKK-β has not been determined yet, therefore structure was generated using homology modelling. Based on the structure of available anti-diabetic drug - chlorpropamide and nateglinide, new ligands have been designed and their ability to inhibit IKK-β has been tested through docking, inhibition, toxicity and drug likeliness studies. We propose that 1-(4-chloro-3-methoxybenzenesulfonyl)-3-propylurea, can be a potential candidate for future drug development to counter drug resistance and provide effective treatment for non-insulin dependent diabetes mellitus through IKK-β inhibition.

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