INHIBITION OF SPLEEN TyROSINE KINASE (SYK) IN SILICO BY THIAZOLE DERIVATIVES

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ABSTRACT

Variously substituted thiazoles derivatives showed wide range of biological activity. The computational evaluation of the substituted thiazole containing derivatives as SYK inhibitor was performed. By using online servers applying different approaches the Tyrosine protein kinase (SYK) was identified as target for variously substituted thiazole containing derivatives. The molecular descriptor analysis was performed to identify drug likeness of the thiazole derivatives by Lipinski’s rule of five. Then docking analysis was performed by PyRx software using AutoDock vina 4. Derivatives showed comparatively significant binding energy value followed Lipinski’s rule. The study provides the base for further in vitro and in vivo study of substituted azoles containing compounds as SYK inhibitor and proposed drug to be used for allergic diseases.

Keywords: Tyrosine protein kinase SYK, thiazole derivatives, Molecular docking, Molecular descriptor, Lipinski’s rule of 5.

INTRODUCTION

In the past decade, significant progress in understanding the mechanism of pathogens and their roles in relevant cells involved in allergic and asthma diseases3. Asthma and allergic disorders presently become high interest for drug discovery. Drugs, like bronchodilators or anti-inflammatory agents, are at present available to extravgance either acute or continual symptoms of asthma. While these therapies provide effective and safe treatments for quick relief of asthma, many of these treatments for persistent symptoms have either side effects, and show low efficacy in refractory patients2. Tyrosine kinases recruited to a receptor following hormone binding are receptor-associated tyrosine kinases and are involved in a number of signaling cascades, in particular those involved in cytokine signaling. One such receptor-associated tyrosine kinase is JAK STAT pathway. Activation of spleen tyrosine kinase (SYK) was shown to be the earliest detectable signaling response to cross-linking of the high affinity IgE receptor (FceRI) cross-linking on mast cells, an event which subsequently leads to mast cell degranulation1,4. Syk inhibition have therapeutic value in the treatment of various diseases like rheumatoid arthritis and other forms of arthritis, systemic lupus erythematosus, autoimmune cytopenias, allergic and autoinflammatory diseases6,7 and acts tumor suppressor in breast cancer8. This importance makes SYK a therapeutic target for inflammatory diseases so, many pharmaceuticals companies, as well as many academic institutions, are interested to involve in the development of small-molecule inhibitors of SYK.

In present, various approaches used to identify the potential target (protein) for the proposed thiazole derivatives. The proteomic approach which compares the protein expression profiles for a given cell or tissue in the presence or absence of the compounds and thus identifies the target. In current scenario the in silico drug target profiling is rising as an effective alternative for an high throughput and laborious in vitro screening9, 10. In addition, an activity referred to as a drug repurposing in which the old drugs are screened for a new target is possible by this approach9,11. We used on line web servers to identify the potential protein targets for proposed variously substituted thiazole derivatives.

MATERIAL AND METHODS

Docking investigation

Ligand Structure Preparation

The substituted proposed thiazole derivatives used as ligand drug data set. ChemSketch12, the chemically intelligent drawing interface freeware (http://www.acdlabs.com/download) was used to draw the structures of substituted thiazole (Figure 1), followed by generation of structure in PDB and SDF format using “On line SMILES translator and structure file generator” (http://cactus.nci.nih.gov/services/translate/) utility which relies on CACTVS technology and utilizes the algorithm of program COOidINAteS (CORINA)13,14 to generate 3D atomic coordinates of a molecule.

Potential Target Identification

To identify the potential targets (protein), we used three online web servers for the variously substituted thiazoles derivatives. Initial screening was done by predicting the bioactivity score at the molinspiration server provided at www.molinspiration.com. The server calculates the drug likeness score towards GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets using sophisticated Bayesian statistics method15. For further precise identification of the target protein the variously substituted thiazoles derivatives files in SDF format were submitted to the Pharmmapper (http://59.78.96.61/pharmmapper/index.php) and ReverseScreen3D (http://www.modelling.leeds.ac.uk/ReverseScreen3D/index.html) servers. The PharmMapper server uses the reverse pharmacophore approach. The output of a PharmMapper run is demonstrated in the form of a ranked list of hit target pharmacophore models that are sorted by fit score in descending order16. The ReverseScreen3D server uses reverse virtual screening (VS) method called ReverseScreen3D. The method uses a 2D fingerprint-based method to select a ligand template from
each unique binding site of each protein within a target database. The target database contains only the structurally determined bioactive conformations of known ligands. The 2D comparison is followed by a 3D structural comparison to the selected query ligand using a geometric matching method, in order to prioritize each target-binding site in the database\textsuperscript{17}. The output is in the form of a list of the 2D and 3D scores in descending order.

**Protein Molecule Preparation**

After the initial screening of the derivatives for the potential target identification the tyrosine protein kinase SYK was considered as best target and used for further analysis. The coordinate for the crystal structure of tyrosine protein kinase SYK was downloaded (PDB id 3EMG)\textsuperscript{18} from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The PDB file was prepared by only selecting the chain A of the protein molecule and saving the file in the absence of the ligand using the Swiss-PDB viewer\textsuperscript{19}. Also the ligand i.e. 4-thiazoyl-2-phenaminopyrimidine was selected and saved as separate PDB file to be used as reference ligand. The new tyrosine protein kinase SYK protein file (PDB), the reference ligand file (PDB) and the variously substituted thiazole derivatives PDB files were used for further molecular docking analysis.

**Docking Analysis**

Molecular docking involves the rapid computational assessment of most favorable interacting regions between two different molecules\textsuperscript{20}. For docking study of variously substituted thiazoles derivatives in tyrosine protein kinase SYK the PyRx virtual screening software for computational Drug Discovery was used. It uses a large body of already established open source software such as Autodock 4\textsuperscript{21} for docking study. The binding energies were obtained for the substituted thiazole derivatives and the reference ligand. The binding pose (figure 2) showing best score were visualized in PyMOL\textsuperscript{22} and Ligplus.

**Molecular Properties**

The molecular properties were calculated on basis of simple molecular descriptors used by Lipinski’s rule of five\textsuperscript{23}. The five properties consist of molecular weight, hydrogen donor; acceptors, LogP, and total polar surface area (TPSA) which were calculated using the online cheminformatics tool molinspiration (http://www.molinspiration.com).

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**Figure 1: Structure of Thiazole derivatives**

1. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
2. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
3. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
4. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
5. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
6. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
7. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
8. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
9. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
10. \(\text{H}_2\text{C} - \text{S} - \text{N} - \text{C}_\text{H}_3\)
Fig 2A, 3A, 4A, 5A
Make size of fig like 1A: Thiazole derivatives interaction with Tyrosin protein kinase

Fig. 1A

Fig. 2A

Fig. 3A
Table 1: Potential Target Identification for thiazole derivatives

<table>
<thead>
<tr>
<th>Thiazole derivatives</th>
<th>Molinspiration Bioactivity Score Kinase Inhibitor</th>
<th>Pharmapopper Score (Tyrosine protein Kinase)</th>
<th>Reverse screen3D Score (Tyrosine Protein Kinase)</th>
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<td></td>
<td>Fit Score</td>
<td>Normalized Fit Score</td>
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<td>3D Score</td>
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<td>Reference ligand</td>
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Table 2: Binding Energy and Molecular Descriptor Study for thiazole derivatives

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<tr>
<th>Thiazole derivatives</th>
<th>Binding Energy(kcal/mol)</th>
<th>Log P Value</th>
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</table>
RESULT AND DISCUSSION

In current study the potential target identification for variously substituted thiazoles derivatives was done and the target was found to be tyrosine protein kinase SYK. Further evaluation of binding efficiency between tyrosine protein kinase SYK and variously substituted thiazoles derivatives was done by using computational approach. The derivatives showed quality binding energy scores which illustrate that they have significant affinity towards tyrosine protein kinase SYK.

Potential Target Identification

Potential target identify using three online servers for variously substituted thiazole derivatives and the scores were obtained as shown in Table 1. The Molinspiration bioactivity score was seen to be significant towards the kinase inhibitor. The results obtained from the Phrammapper server showed the high fit scores and rank above 50 for variously substituted thiazoles derivatives as ligand of the tyrosine protein kinase SYK. Screening outcome of ReverseScreen3D also showed top rank and high scores (2D and 3D) for the tyrosine protein kinase SYK (PDB id: 3EMG). Thus the tyrosine protein kinase SYK (PDB id: 3EMG) was considered to be potential target for variously substituted thiazole derivatives and used for further molecular docking analysis.

Docking and Molecular Descriptor analysis

Docking analysis was performed by PyRx virtual screening software using Autodock 4. The binding energies calculated are as shown in Table 2. Thiazole derivatives 6, 7 and 8 showed significant binding energy values compared to the reference ligand than other thiazole derivatives. The molecular descriptor study was explained by "Lipinski’s rule of five" using the Molinspiration server. Lipinski’s rule of Five is a rule of thumb to evaluate drug likeness, the rule was formulated by Christopher A Lipinski et al., and the rule describes molecular properties important for explaining a drug’s pharmacokinetics profile in the human body, including their absorption, distribution, metabolism, and excretion (“ADME”).

Discussion of docking study

Derivative 6 seems to be stabilized mainly via hydrophobic contacts with residues in active site of SYK. Terminal aniline moiety is stacked in a hydrophobic pocket formed by residues Met448, Met424, Phe513, Ser511, and Glu420. Such a stacking hydrophobic contact is experimentally found to be significant is stabilization of SYK. Asp512 shares a hydrogen bond via its OD2 atom with N2 nitrogen atom of thiazole moiety from Derivative 6. Such polar interaction with Asp512 is experimentally verified to stabilize inhibitor-SYK complex. N1 nitrogen atom of derivative 6 seems to hydrogen bond with backbone oxygen atom of Ala451. This hydrogen bond is also found to be essential for stabilization of experimental inhibitor-SYK complex. Fluoro phenyl fragment of derivative 6 is occupied in hydrophobic pocket formed by Gly454, Met452, Met450 residues (Figure 1A). Its binding energy (-7.49 Kcal/mol) is found to be effective than experimentally verified ligand 4-thiazolyl-2-phenylaminopyrimidine indicating its better potential as anti-SYK compound.

From fig. 2A it is clear that derivative 7 attains stable conformation due to placement of its 2-methyl aniline fragment in the hydrophobic pocket of the SYK. Its 2-methyl aniline moiety is fixed in the hydrophobic residues Gly454, Leu453, and Glu452. Such conformation is experimentally established as an essential stabilizing force for SYK-Inhibitor complexes. Backbone oxygen of the Leu377 interacts with N4 and N1 nitrogen atom of the thiazole moiety and Asp512 is hydrogen bonded through OD2 oxygen with N2 nitrogen of thiazole of derivative 7. Such type of hydrogen bonding is essential for stabilization of derivative 7 in SYK complex. 4-Fluoro phenyl fragment of derivative 7 is stabilized in the cag of hydrophobic pocket formed by Glu420, Met448, Lys402, Ser511, Arg498 residues of SYK. Binding energy of derivative 7 with SYK complex is -7.16 kcal/mole is better than the reference molecule and can emerge as an efficient SYK inhibitor.

From fig.3A it seems that derivative 8 is stabilized via contacts made of residues like Glu452, Gly454, Leu453 and Pro455 from SYK with 3methylamino moiety of derivative 8. Backbone oxygen of Leu377 is in hydrogen bonding with nitrogen atom N4 of terminal 3methyl aniline moiety of derivative 8. Backbone oxygen of Leu377 is in hydrogen bonding with nitrogen atom N4 of terminal 3methyl aniline moiety of derivative 8. The residues Arg498, Gly378, Glu420, Ser511, Lys402 and Met448 makes hydrophobic pocket of SYK and fluoro benzyl moiety is well stabilized in hydrophobic region. Oxygen OD2 of Asp512 form hydrogen bond with nitrogen of thiazole moiety and stabilizes derivative 8. Such polar and lipophobic contacts are experimentally verified to impart stability to SYK-inhibitor complexes.

From fig.4A it indicate that derivative 9 attained stability due to the hydrophilic interaction of the terminal oxygen of residue Ala451 with N1 and N2 atom of thiazole moiety also 4-fluoro phenyl fragment of derivative 9 formed hydrogen bonding with terminal oxygen of residue Lys402. 4-methyl aniline moiety form stable conformation in the hinge region of residue Lys377, Glu452, Met450, Gly454 and other terminal of derivative 9 in contact with the non polar pocket of the residue Asp512, Glu420, Met448, Ser511, Ala404, and Leu501. Such polar and non polar interactions with SYK are experimentally conformed to convey stability to SYK-inhibitor complexes.

From fig.5A it seems the oxygen of terminal 4- methoxy aniline moiety of derivative 10 gives hydrophilic hydrogen bonding with the residue of Asp512 through nitrogen atom and makes its stable conformation. The hydrophobic residues of SYK like Ser511, Glu420, Met424, Met448, Lys402 and Leu501 helps stability to derivative 10. Polar Residue Ala451 form hydrogen bonding with N4 atom of the thiazole moiety and Met450, Gly454, Glu452, Leu377 and Leu655 makes hydrophobic surrounding for the terminal 4-fluorophenyl moiety of derivative 10. Such interactions with SYK found to be effective than experimentally verified indicates its better potential as anti-SYK compound.

CONCLUSION

In present study the thiazole derivatives was identified to be tyrosine protein kinase(SYK) inhibitor and binding energy values were significant which showed the prediction to be precise. So indeed the study gives a platform for the further in-vitro and in-vivo analysis of the inhibitor of the tyrosine protein kinase.

REFERENCES


22. The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.


