Short Communication

Molecular docking studies of 1,3,4-thiadiazoles as myeloperoxidase inhibitors

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ABSTRACT

Background: Myeloperoxidase (MPO) is a heterodimeric, cationic and glycosylated haeme enzyme which gets released under increased oxidative stress producing neutrophil oxidant, hypochlorous acid having the capacity to modify various biomolecules by chlorination and/or oxidation of sulfhydryl groups in proteins causing their inactivation and promoting inflammatory tissue damage. Different levels of hypochlorus acid are used as a trait marker for prescribing the disorders e.g. atherosclerosis, rheumatoid arthritis, lung cancer, Immuno-reactivity.

Methods: Mini library of 22500 2,5disubstituted 1,3,4 thiadiazoles were docked with Myeloperoxidase in order to identify the potent inhibitor against the enzyme. The chemical nature of the protein and ligands greatly influence the performance of docking process. Keeping this fact in view, critical evaluation of the performance was performed by GLIDE by HTVS, SP and XP. The ADME parameters by QIKPROP and protein-ligand binding free energies were calculated using the Prime/MM-GBSA module of Schrödinger.

Results: Both hydrogen bonding and hydrophobic interactions contributed significantly for its ligand binding and core influence the target site through prominent hydrophobic and charged interaction with the backbone and side chain residues in the target site that improves the affinity of the molecule. The compound selected as potent inhibitor is having minimum binding affinity, maximum GScore and minimum FlexX energy. The amino acids residues ASP98, ASP94, THR100 and GLU 102 in the MPO gene domain active site form hydrogen bonds with the ligand. Compounds 3350-5150 showed better interaction with haeme enzyme for further understanding of structures, reliability and Biomolecularactivity in connection with oxidative stress induced disorders.

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1. Background

In humans, oxidative stress may be involved in many diseases including atherosclerosis.1, Parkinson’s disease2, heart failure, myocardial infarction3; Alzheimer’s disease4, schizophrenia, cancer5,6 etc. Myeloperoxidase is a haeme enzyme abundant in granules of human inflammatory cells. The enzyme MPO as the prominent generator of reactive oxidizing species in neutrophils uses H2O2 and (pseudo) halides mostly chloride ion due to its increased concentration in body fluids to produce hypo (pseudo) halous acids (HOX, HOSCN). Myeloperoxidase gets released outside the phagocytes producing the most powerful and stable neutrophil oxidant, hypochlorous acid. This production of HOCl contributes to tissue damages. HOCl has the capacity to modify various biomolecules by chlorination and/or oxidation of sulfhydryl groups in proteins causing their inactivation. MPO is implicated in a growing number of diseases like atherosclerosis, rheumatoid arthritis, lung cancer and many others.HOCl has the capacity to modify various biomolecules by chlorination and/or oxidation of sulfhydryl groups in proteins causing their inactivation.7,8 HOCl act as an immunological tool to identify chlorinated biomarkers in kidney disease, ischemia reperfusion, Parkinson’s disease and atherosclerosis.9,10,11
In the present study, combinatorial technology was applied to design and generate a small library consisting of 22,500 molecules 1, 3, 4- thiadiazole scaffold. Myeloperoxidase was selected as target for identification of lead molecules with good ADME properties and effective binding affinity with target enzyme myeloperoxidase.

2. Materials and Methods

Schrodinger software version was used for the docking studies. For the determination of protein–Ligand binding affinities and scoring function GLIDE 9.1 (Grid Based Ligand Docking with Energies) XP (Extra Precision) docking protocol was used. Combinatorial technology was applied to design and generate a small library consisting of 22,500 molecules 1, 3, 4- thiadiazole scaffold. Myeloperoxidase was selected as target for identification of lead molecules with good ADME properties and effective binding affinity with target enzyme myeloperoxidase.

2.1. Myeloperoxidase

(4C1M) -Resolution of Myeloperoxidase (4C1M) selected was 2.00 Å and the R value and r free value was found to be (0.225, 0.278). The target protein was prepared and viewed through the ramachandran plot which shows clearly for all the essential aminoacids to be in the favourable region (Red colour), some in slightly favourable region (yellow) and none in unfavourable region (White).

As per the literature of this particular pdb file 4CIM gives information that the preferable conserved H-bonding interactions of inhibitors with hinge region residues Lys 308c, Phe 407, Arg 239 and Gln 91a are observed in most of the inhibitor-myeloperoxidase complex structures.

2.2. Ligand database preparation

The structure of ligand molecules was built in the panel of Maestro and store (in. mae) format by using the build tool and the fragments which had been used were shown in the figure 2. ADME studies: A set of ADMET-related properties (a total of 50 molecular descriptors) Table 1 was calculated by using the QikProp program (Schrödinger 2011d) running in normal mode.

2.3. Ligand receptor docking and predicting activity

After the preparation of protein, the prepared ligands were docked with the respective proteins. The ligands Database which consist of 22500 molecules which had been already prepared by ligprep were filtered by qikprop and first initially docked by using Glide in HTVS (high throughput virtual screening) mode and Standard mode (sp).

2.4. Specifying the Receptor Grid

The receptor grid image of myeloperoxidase with its active site is shown below.

After investigating the results of docking study and predicted activity top 20 molecules were selected and then the common scaffold was identified using the thiadiazole scaffold. These molecules were than tagged as 3205 to 5150. The molecules molecular name was again investigated as a literature review to find out the information. 5150 tagged compound was found to have effective binding affinity.

2.5. Bind site analysis

Active sites or binding sites for enzymes were predicted from a pictorial database of 3D structures in the protein data bank (PDB sum), and Q-Site Finder software from university of Leeds Bioinformatics was used for ligand binding site prediction. In that 6 sites where found active (1 for ligands and 5 for metals). So it was decided to keep all the amino acids in the active site of the enzyme.

3. MM-GBSA Calculations of Protein-Ligand Binding Free Energies

3.1. Molecular mechanics/generalized born surface area (MM/GBSA)

In this study, computational methods were applied to identify binding site interaction details between thiadiazoles scaffold and myeloperoxidase. The protein-ligand binding free energies during the last 2 ns were calculated using the Prime/MM-GBSA module of Schrödinger suite to get the averaged binding property. The binding free energy ΔGbind was estimated using the equation:

3.2. ΔGbind = gcomplex — (gprotein + gligand) (2)

Where G complex is the optimized free energy for the complex, Gprotein and Gligand are the optimized free energy for the free protein and free ligand, each energy term was calculated by a combination of molecular mechanics energy, implicit solvation energy and surface area energy. Residues in binding pockets of the protein were treated as flexible and the ligand partial charges were assigned by the initial Charm charges. MM/GBSA has been successfully applied to various protein-ligands but their performance is system dependent.

4. Results

In this work, library of thiadiazoles derivatives with modifications in the 2nd position and amino group of 5th position were used for the study. QikProp predicted physically significant descriptors and pharmaceutically relevant properties of organic molecules, in batches.
Fig. 1: Receptor site of Myeloperoxidase (4C1M) & Ramachandran plot of Myeloperoxidase receptor pdb file (4C1M)

Fig. 2: Heterocyclic nucleus used as Reagent 1, Reagent 2 & toxicity filters in the corelibrary generation

Fig. 3: Receptor grid image of myeloperoxidase receptor (4C1M)
Fig. 4: Compound 5150 in mesh form. (A) 2D ligand interaction showing interaction of 5150 with myeloperoxidase receptor amino acid residue Arg 30, Asp 98 and Phe 99. (B) NH amine in ligand 5150 forming H-bond with the O in Carboxyl group of Asp 98. Aromatic ring of ligand forming hydrophobic bond with aromatic ring in Phe 99 and C-Chain in Arg 30. (D) Compound 5150 been docked with the myeloperoxidase receptor.

Fig. 5: Visualization of Prime energy and ΔG binding energy of compound 5150 with enzyme myeloperoxidase.
Table 1: ADME parameters prediction of the screen1, 3, 4-thiadiazoles

| ID  | RO3 | QPPP | CaCo | MDCK | QPLog KHSa | Jm | Metabolism | QPlogHERG |
|-----|-----|------|------|------|------------|----|------------|------------|
| 3205| 0   | 351.307 | 216.715 | -0.68 | 0.3 | 3   | 3         | -5.037     |
| 3350| 0   | 529.258 | 450.343 | -0.548 | 1.484 | 1           | 3         | -5.802     |
| 3500| 1   | 3.066  | 1.911 | -1.184 | 0.002 | 2   | 3         | -3.407     |
| 3650| 0   | 853.918 | 571.97 | 0.045 | 0.031 | 4   | 3         | -5.266     |
| 3800| 0   | 842.567 | 748.567 | 0.171 | 0.065 | 3   | 3         | -5.884     |
| 3950| 1   | 4.604  | 2.675 | -0.68 | 0    | 3   | 3         | -3.572     |
| 4100| 1   | 1377.079 | 1147.187 | 0.412 | 0.005 | 4   | 3         | -6.418     |
| 4250| 1   | 1366.183 | 1261.64 | 0.606 | 0.009 | 2    | 7         | -7.097     |
| 4400| 1   | 6.843  | 4.812 | -0.301 | 0    | 3   | 4         | -6.726     |
| 4550| 0   | 1225.518 | 807.363 | 0.281 | 0.027 | 6   | 4         | -5.557     |
| 4700| 0   | 1836.642 | 1734.43 | 0.42 | 0.112 | 2    | 7         | -7.097     |
| 4850| 1   | 11.074 | 7.64  | -0.508 | 0.001 | 5   | 3         | -3.877     |
| 5000| 1   | 1460.013 | 986.66 | 0.705 | 0.006 | 4   | 4         | -7.493     |
| 5150| 1   | 1452.113 | 1091.19 | 0.855 | 0.016 | 2   | 3         | -3.124     |
| 5300| 1   | 12.396 | 8.639 | -0.301 | 0    | 3   | 5         | -7.889     |
| 5450| 0   | 3148.614 | 2775.842 | 0.193 | 0.759 | 5   | 5         | -5.435     |
| 5600| 0   | 3136.279 | 3080.97 | 0.317 | 1.996 | 3   | 6         | -6.141     |
| 5750| 1   | 18.105 | 14.099 | -0.563 | 0.008 | 4   | 8         | -7.097     |

Range Max 3 <25 p, >500 great <25 p, >500 great -1.5 to +1.5 1-8 Concern below-7

Six targets of binding sites on the crystallographic structure of the enzyme have been examined for ligand-based docking program. The ligands are screened for their ability to dock within the active site of the enzyme. Instead extra precision mode(XP) were used. After analyzing the different docking interactions of ligands, the compounds namely 3350, 3500, 3950, 4400, 4850, 5300 and 5750 showed fairly better interaction with Myeloperoxidase with the more negative G-Score value than the other drug molecules. The docking scores summarized in with glide score ranging from -5.3 to -1.3. The free energy of binding of various conformers of ethambutol with the receptor was calculated employing Prime MM-GBSA approach.

5. Discussion

The designed library was filtered for drug like properties with the help of qikprop. The pharmacokinetic properties (ADMET) were predicted and the results are tabulated (table 1). In drug likeliness assessment 50 % showed lead like
Table 3: Free energy of binding of various conformers of thiaodiazole scaffold with the receptor myeloperoxidase (4C1M)

| ID   | Prime MMGBSA Complex Energy | Prime MMGBSA Ligand Energy | Prime MMGBSA Receptor Energy | Prime MMGBSA DG bind (ΔGbind kcal/mol) | Prime MMGBSA DG bind Coulomb | Prime MMGBSA DG bind vdW |
|------|----------------------------|----------------------------|-----------------------------|----------------------------------------|----------------------------|-------------------------|
| 3205 | -3895.3                    | -16.752                    | -3843.57                    | -30.534                                 | -25.135                    | -23.549                 |
| 3350 | -3886.6                    | -4.367                     | -3843.57                    | -32.682                                 | -17.011                    | -21.644                 |
| 3500 | -3905.4                    | -20.482                    | -3843.57                    | -35.37                                  | -46.125                    | -19.964                 |
| 3650 | -3919.1                    | -32.299                    | -3843.57                    | -37.26                                  | -16.817                    | -24.038                 |
| 3800 | -3927.5                    | -35.14                     | -3843.57                    | -42.838                                 | -3.678                     | -27.52                  |
| 3950 | -3925.7                    | -54.865                    | -3843.57                    | -32.989                                 | -21.636                    | -20.985                 |
| 4100 | -3870.1                    | 23.553                     | -3843.57                    | -44.136                                 | -16.683                    | -30.219                 |
| 4250 | -3874.6                    | 18.952                     | -3843.57                    | -44.051                                 | -9.442                     | -25.123                 |
| 4400 | -3891.6                    | 2.699                      | -3843.57                    | -44.78                                  | -12.885                    | -33.611                 |
| 4550 | -3929.8                    | -41.175                    | -3843.57                    | -39.069                                 | -12.601                    | -27.313                 |
| 4700 | -3931.2                    | -48.298                    | -3843.57                    | -33.319                                 | -16.701                    | -18.951                 |
| 4850 | -3943.1                    | -63.608                    | -3843.57                    | -35.841                                 | -36.428                    | -20.08                  |
| 5000 | -3880                      | 7.343                      | -3843.57                    | -37.575                                 | -11.708                    | -31.894                 |
| 5150a| -3896.6                    | 1.688                      | -3843.57                    | -48.725                                 | -9.844                     | -29.853                 |
| 5300 | -3925.7                    | -32.856                    | -3843.57                    | -43.333                                 | -31.448                    | -23.514                 |
| 5450 | -3894.5                    | -13.611                    | -3843.57                    | -31.314                                 | -15.97                     | -21.186                 |
| 5600 | -3907.7                    | -17.733                    | -3843.57                    | -40.401                                 | -11.88                     | -23.77                  |
| 5750 | -3923.2                    | -34.84                     | -3843.57                    | -38.767                                 | -31.256                    | -22.99                  |

* most stable conformer

and 30% of the screened compounds showed fragment like property. All compounds satisfied the jorgensson rule of 3, fell within the range for Caco-2 permeability test so they are likely to be more orally bioavailable. As MDCK cells serve as good mimic for BBB it was estimated that about 66% of compound had apparent MDCK cell permeability falling within the recommended range for 95% of known drugs. The predicted maximum transdermal rates Jm fell within the predicted range. The plasma protein binding was predicted using logkHSA more than 96% of compounds are compliant to this parameter indicating free circulation within the bloodstream and access to target site. 100% of the compound predicted to undergo the recommended no of metabolism reactions (1-8) with 10% in drug like (5-6), 50% in lead like (3-4) and 40% in fragment like subset. logIC50 values for blockage of HERGk+ channel is >-5. The parameter was found to be recommended range for majority of compounds.

As per the results obtained core influence the target site through prominent hydrophobic and charged interaction with the backbone and side chain residues in the target site that improves the affinity of the molecule. Overall the core had good influence in the target site, and there is large scope for the optimization of the core keeping all the facts in the mind. The major interactions formed by ligands with the receptor myeloperoxidase may be categorized as hydrogen bond, hydrophobic bond, π-π stacking and electrostatic interactions. All the ligands showed selected strong hydrogen bond interactions with the amino acids Arg31, Thr100, Gln91, Glu102, Asp98, Asp94, Hip95, Asp98 and Leu97 of myeloperoxidase. Thr100 forms hydrogen bond with its backbone carbonyl oxygen and NH of ligand. The aromatic nature of the compound showed common Pi-Pi interactions with aromatic ring of Phe99, imidazole ring in Hip95. Overall the hydrogen bond and hydrophobic interactions formed by amino acids Asp98, Asp94, Thr100 and Glu102. Hydrophobic interactions were found with amino acids Pro101, Ala104, Ala105, Phe29, Leu97, Met87, Phe99 and Phe29 are all critical for stabilizing the inhibitors inside the binding pocket of the myeloperoxidase receptor. The thiaodiazole derivatives and its different analogues were found to bind with Myeloperoxidase enzyme. Figure 4 The docking screening was performed by employing the scoring function. The result was based on the score of estimated free energy, inhibition constant, and hydrogen bonding.

The most stable conformer (ΔGbind = -48.725 kcal/mol) of the screened subset of compounds was found to compound 5150. The compound showed that its amine group hydrogen was exposed to the hydrophilic region of receptor indicated in blue colour Figure 5 and formed strong H-Bond interactions with crucial amino acid residue Asp98, at a distance of 1.626 Å. The aryl unsaturated chain of compound 5150 was found to be embedded in the hydrophobic region of the receptor shown as blue colour in the Figure 5 which indicates the probability of binding of compound 5150 is in competition with the natural substrate of the receptor. The other stable conformers from...
the screened subset were found as Compound 3350, 3800, 4100, 4250, 4400, 4700, 5300 and 5600 showing \( \Delta G_{\text{bind}} = \text{kcal/mol} \) binding values in the range of (-33.319 to -48.725)

6. Conclusion

The present work deals with generation of a small combinatorial library of 22500 ligands using virtual screening method of thiadiazole scaffold. Myeloperoxidase was selected as target for the study and the designed ligands were screened for their binding affinity of ligands was calculated by MMGBSA approach and Molecular docking was carried out at three levels (HTVS, SP and XP) and top myeloperoxidase inhibitors were identified On comparing the glide score values, the better interaction was shown by compounds with glide score values-5.3 respectively. Thus by analyzing these datas 1 3 4 thiadiazole derivatives can be considered as a potent inhibitor against the enzyme myeloperoxidase in oxidative stress.

7. Source of Funding

None.

8. Conflict of Interest

None.

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