**Journal of Computational Methods in Molecular Design, 2013, 3 (2):26-31**

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**ISSN : 2231- 3176**
**CODEN (USA): JCMMDA**

# Molecular characterization and identification of copper transport in wilson disease

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**ABSTRACT**

*Wilson's disease or hepatolenticular degeneration in an autosomal recessive genetic disorder in which copper accumulates in tissues. The condition is due to mutations in Wilson disease protein (ATP7B). A special feature of WD is that medical therapy is used to treat presymptomatic as well as symptomatic individuals, and hepatic transplantation can reverse the metabolic abnormality. In the present research work, an attempt has been made to map the binding site residues of ATP7B protein (receptor). The 3D structure of the monomeric protein was not yet not been experimentally elucidated for which the theoretical structure was determined by homology modeling using 2UVC as template and the structure was validated and refined by SAVS and MODELLER. The generated 3D structure of ATP7B protein was solvated at 310k and energy minimized using Gromacs. Similarly, 2D structures of several diuretic compounds were retrieved, refined and energy minimized using Arguslab software. The diuretic compounds were then subjected to analyze their adsorption, distribution, metabolism, excretion and their toxicity properties. This analysis was done using the online server PREADME/TOX. The protein- ligand interactions between the ATP7B protein and the diuretic compounds were analyzed using Autodock - PyRx and the docking models were visualized and analyzed by PyMol. Based on the output of the docking models and the binding energies the best lead compound was determined which can be used for fighting against the Wilson disease.*

**Keywords:** Wilson disease; Homology modelling; Docking.

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## INTRODUCTION

Wilson's disease is a genetic disorder, where the copper gets deposited in tissues. The pathogenic defect in Wilson disease are neurological dysfunction and psychiatric dysfunction and liver disease. This is because mutations occurs in Wilson disease protein (ATP7B). A single copy of abnormal gene occur in 1 in 100 people, which does not shown any symptoms. If both the parents are inheretied with the Wilson disease, it may be carried to their next generation. [1]. It generally appears between the age group of 6 to 20 years, but it is mostly described about old age people. These liver disease based on yellowing of the skin and the eyes turned to white (jaundice), fatigue, loss of appetite and abnormal swelling. It may also include clumsiness, trembling, difficulty, walking, etc... In many people with Wilson disease, were the copper gets deposited in the cornea of eye., which turns from green-to-brownish ring, is called as Kayser-Fleischer ring.[4]. Copper is an essential elements, which acting as a cofactor for number of proteins. In our day today life the average amounts of copper is nearly 2-5 mg/day, the intake is 0.9 mg/day. Most deitry copper ends with excretion. Copper is mainly absorbed in duodenum and proximal small intestine and they are transferred in the portal circulation with the albumin amino acids such as histidine to liver, it may removed through circulation. From these the liver absorb some copper content for the metabolic process, synthesis and provides copper contains protein ceruloplasm, and remaining copper excreated into bile. [3].The protein present in Wilson disease is ATP7B which is denoted as copper transporting P-type ATPase. It is P-type family. These P-type cation transport ATPase family, encodes a protein with various membrane-spanning domains. This protein acts as monomer, copper is exported to their outer cells, such as hepatic copper into bile. There are different encoded isoforms with distinct cellular localizations, has been characterized by various transcriptional splice varients.[5]. The Wilson disease ATPase protein was identified as a copper transporting P-type ATPase by sequence analysis method.

This ATPase protein was well differentiated from other members of P-type ATPase family and was further classified into CPx-type, typeI or heavy meatel P-typr ATPase. [6]. The N-terminus of the CPx-type ATPase protein has an additional pair transmembrane helices and a cytoplasmic metal binding domain which makes these protein family differ from other P-type ATPase. In addition to a pair of cysteines flanking the conserved proline residue in the transduction domain, the histidine and proline residues of the SEHPL sequence motif are highly conserved in heavy metal–transporting ATPases. The most common mutation occur in Wilson disease is histidine residues H1069Q. [2].

## MATERIALS AND METHOD

### HOMOLOGY MODELING

The three dimensional structure of ATPase protein was not been elucidated. The theoritical structure was generated by homology modelling method using Modeller9v11(*Sali et al 1993*). A comparative protein structure modelling program is derived from the alignment which includes deriving target sequences, extracted from alignment with template structures. The alignment file was generated from the CLUSTALW and the position of the amino acid residues for the template and target files were provided in the alignment file. For the target, alignment position was specified from the CLUSTALW alignment and for the template file the position was specified from the atom file. After the files were prepared, modeller was runned. The quality check for the final structured was performed by using SAVS server. The Ramachandran plot analysis indicated that of the residues were in allowed regions. From that disallowed region were loop refined.

### MOLECULAR DYNAMICS – GROMACS

The 3D structure were generated by using modeller9v11, then it is stimulated by molecular dynamics using GROMACS and they are calaulated by using force fields. These models were energy minimized in vaccum at 310k to obtain their global energy minima in isolation because these are necessary to obtain a entire protein. The modelled receptor is solvated in water and the same is then energy minimized. A major component of the cytosolic fluid is water. Hence, minimizing the structure in water should give an idea of the native conformation of the protein in this environment. GROMACS is an engine to perform molecular dynamics and energy minimization.

### ADMET PREDICTION

ADME means absorption, distribution, metabolism and excretion, which are major parts of pharmacokinetics,nowadays ADME properties are important conditions to choose compounds as drug. And it is also based on the Toxicity prediction that means desiging drugs with the consideration of their toxicity is very important. PreADMET predicts mutagenicity and carcinogenicity of a compounds, helps to avoid toxic compound.

### MOLECULAR DOCKING

The molecular docking is based on binding pattern of ATP7B protein with various diuretic compounds. The active site were identified and the interaction between the receptor and diuretic compounds is identified with the help of molecular viewer,from these docking results were obtained with binding values.

## RESULTS AND DISCUSSION

### Modelling of protein ATP7B:

The 3D structure of ATP7B protein have not yet been reported. The three dimensional structure of ATP7B protein have generated by homology modelling using 2UVC as a template with 82% identity.

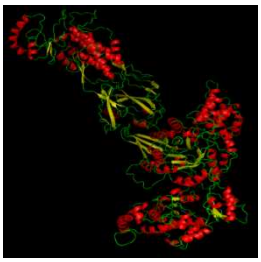


**Fig 1: Structure of ATP7B Protein**

### Lead optimization study

The objective is to optimize lead compounds i.e. new analogs with improved potency, reduced off-target activities, and physiochemical/metabolic properties suggestive of reasonable in vivo pharmacokinetics. Lipinski's Rule of five

is a rule of thumb important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity. Lipinski's rule of 5 helps in distinguishing between drug like and non drug like molecules. These are the following steps:

- Molecular mass less than 500 Dalton.
- High lipophilicity (expressed as LogP less than 5).
- Less than 5 hydrogen bonds donors.
- Less than 10 hydrogen bond acceptors.
- Molar refractivity should be between 40-130.

**Table 1: List of compounds satisfying Lipinski's Rule**

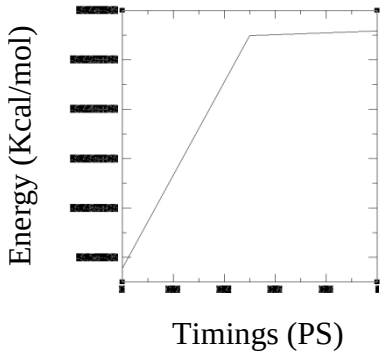
| S.No | Drug Name | CID | LIPINSKI'S RULE OF FIVE | | | | |
|---|---|---|---|---|---|---|---|
| | | | LogP | H Donar | H Acceptor | Mol.Wt | Mol Ref |
| 1 | Alfalfa | 170570 | 3.12 | 1 | 4 | 286.327 | 47.93 |
| 2 | Ginkgo | 16219435 | -2.370 | 3 | 10 | 424.402 | 148.83 |
| 3 | Parsely | 10659 | 2.423 | 0 | 4 | 222.24 | 36.936 |
| 4 | Cincletanine | 54910 | 2.823 | 1 | 3 | 261.708 | 42.354 |
| 5 | Goldenseal | 969516 | 2.303 | 2 | 6 | 368.385 | 93.066 |
| 6 | Dandelion | 16180 | 2.524 | 1 | 3 | 221.039 | 46.933 |
| 7 | Thiophenalacetylene | 164797 | 4.331 | 0 | 0 | 198.29 | 0.0 |
| 8 | Zigiberofficinals | 6850760 | 3.217 | 2 | 4 | 294.391 | 66.761 |
| 9 | Didanosine | 50599 | -0.947 | 2 | 7 | 236.231 | 93.042 |
| 10 | Furosemide | 3440 | 1.77 | 4 | 7 | 330.749 | 122.63 |
| 11 | Bumetamide | 2471 | 3.448 | 4 | 7 | 364.423 | 118.72 |
| 12 | Ethacrynic acid | 3278 | 3.436 | 1 | 4 | 303.141 | 63.604 |
| 13 | Torsemide | 41781 | 2.602 | 3 | 7 | 348.428 | 100.18 |
| 14 | Chlorothiazide | 2720 | 0.021 | 3 | 7 | 295.729 | 118.69 |
| 15 | Hydrochlorothiazide | 3639 | -0.06 | 4 | 7 | 297.745 | 118.36 |
| 16 | Benzothiazide | 2343 | 2.11 | 3 | 7 | 431.948 | 118.69 |
| 17 | Hydroflumethiazide | 3647 | 0.157 | 4 | 7 | 331.297 | 118.36 |
| 18 | Acetazolamide | 1986 | -1.46 | 3 | 7 | 222.251 | 60.477 |
| 19 | Spironolactone | 5833 | 3.028 | 0 | 4 | 416.583 | 107.45 |
| 20 | Asteraceae | 114703 | -0.151 | 0 | 3 | 232.279 | 35.539 |
| 21 | Chlorothalidone | 2732 | 1.222 | 4 | 6 | 338.772 | 109.49 |
| 22 | Methyclothiazide | 4121 | 0.788 | 4 | 7 | 360.244 | 109.57 |
| 23 | Bendroflumethiazide | 2315 | 1.949 | 4 | 7 | 421.422 | 118.36 |
| 24 | Metalazone | 4170 | 2.771 | 3 | 6 | 365.842 | 92.501 |
| 25 | Polythaizide | 4870 | 1.623 | 3 | 7 | 439.89 | 109.57 |
| 26 | Quinethazone | 6301 | 0.931 | 4 | 6 | 289.744 | 101.29 |
| 27 | Trichlormethazide | 5560 | 1.113 | 4 | 7 | 380.662 | 118.36 |
| 28 | Aldosterone | 5839 | 1.158 | 2 | 5 | 360.45 | 91.669 |
| 29 | Dichloropheamide | 3038 | 0.537 | 4 | 6 | 305.164 | 102.33 |
| 30 | Etozoline | 5743585 | 1.436 | 0 | 5 | 284.381 | 49.852 |
| 31 | Muzolimine | 41386 | 2.346 | 2 | 4 | 272.135 | 58.696 |
| 32 | Tienilic | 38409 | 3.806 | 1 | 4 | 331.176 | 63.604 |
| 33 | Piretamide | 4849 | 2.657 | 3 | 7 | 362.407 | 109.93 |
| 34 | Mebutizide | 71652 | 2.298 | 3 | 7 | 381.907 | 118.36 |
| 35 | Clopamide | 2804 | 0.991 | 4 | 6 | 345.852 | 92.501 |
| 36 | Mefruside | 4047 | 1.371 | 4 | 7 | 382.891 | 106.77 |
| 37 | Clofenamide | 69594 | 0.991 | 3 | 6 | 270.719 | 120.33 |
| 38 | Meticrane | 4165 | 0.803 | 4 | 5 | 275.351 | 94.307 |
| 39 | Xipamide | 26618 | 2.547 | 2 | 6 | 354.815 | 109.49 |
| 40 | Indapamide | 3702 | 2.455 | 4 | 6 | 365.842 | 92.501 |
| 41 | Chorexolone | 16473 | 2.144 | 2 | 5 | 328.821 | 80.474 |
| 42 | Fenquizone | 68548 | 1.647 | 4 | 6 | 337.788 | 101.29 |
| 43 | Meralluride | 101626 | -1.661 | 3 | 7 | 431.818 | 104.72 |
| 44 | Mersalyl | 443130 | 0.416 | 2 | 6 | 466.863 | 84.865 |
| 45 | Theabromine | 5429 | -0.951 | 1 | 6 | 180.167 | 72.693 |
| 46 | Eplerenone | 5282131 | 1.744 | 0 | 6 | 414.498 | 82.209 |
| 47 | Canrenone | 13789 | 2.026 | 0 | 3 | 340.463 | 43.376 |
| 48 | Mozavaptan | 119369 | 4.706 | 1 | 5 | 427.548 | 52.645 |
| 49 | Tolvaptan | 216237 | 40938 | 2 | 5 | 448.95 | 69.635 |

The compounds which does not satisfy Lipinski's Rule are Amiloride,Triamterenas,Mannitol, Benzamil, Echinacea and Trapterine.

**Energy minimization of diuretic compounds:**
The physical, chemical and biological properties of a molecule depend upon the conformations adopted by molecules. Hence the compounds which has satisfied the Lipinski rule of 5 were energy minimized and conformed with stable conformation with the least energy. These are the diuretic compound, Alfalfa, Ginkgo, Parsely, Cincletanine, Goldenseal, Dandelion, Thiophenalacetylene, Didanosine, Furosemide,Bumetamide, Ethacrynic acid,Torsemide,Chlorothiazide,Hydrochlorothiazide,Benzothiazide,Hydroflumethiazide,Acetazolamide,Spironolactone,Asteraceae,Chlorothalidone,Methyclothiazide,Bendroflumethiazide, Metalazone, Polythaizide, Quinethazone, Trichlormethazide,Aldosterone, Dichloropheamide, Etozoline, Muzolimine, Tienilic, Piretamide, Mebutizide, Clopamide, Mefruside, Clofenamide,Meticrane, Xipamide, Indapamide, Chorexolone, Fenquizone,Meralluride, Mersalyl, Theabromine, Eplerenone,Canrenone,Mozavaptan and Tolvaptan as its stable conformation.

**Molecular Dynamics of ATP7B protein:**
The receptor (ATP7B protein) is solvated in water and the same is then energy minimized. A major component of the cytosolic fluid is water. In view of that, water salvation is done because HTT is present in the cytoplasm. Hence, minimizing the structure in water should give an idea of the native conformation of the protein in its natural environment.

The models are minimized in vaccum at 310K to obtain a their global energy minima in isolation because these are necessary to obtain a better model of entire protein.




Total energy of ATP7B protein after energy minimization is = -6.42171e+06

**Fig 2: Energy Graph of ATP7B Protein**

**ADMET PREDECTION**
The diuretic compounds such as Alfalfa, Ginkgo, Parsely, Cincletanine, Goldenseal, Dandelion,Thiophenalacetylene,Didanosine,Furosemide,Bumetamide,Ethacrynicacid,Torsemide,Chlorothiazide,Hydrochlorothiazide,Benzothiazide,Hydroflumethiazide,Acetazolamide,Spironolactone,Asteraceae,Chlorothalidone,Methyclothiazide,Bendroflumethiazide,Metalazone,Polythaizide,Quinethazone,Trichlormethazide,Aldosterone,Dichloropheamide,Etozoline,Muzolimine,Tienilic,Piretamide,Mebutizide,Clopamide,Mefruside,Clofenamide,Meticrane,Xipamide,Indapamide,Chorexolone,Fenquizone,Meralluride,Mersalyl,Theabromine,Eplerenone,Canrenone,Mozavaptan and Tolvaptan were then subjected to analyze their adsorption, distribution, metabolism, excretion and their toxicity properties. This analysis was done using the online server PREADME/TOX.

**MOLECULAR DOCKING:**
Mapping the binding patterns of ATP7B protein (Receptor) with the various diuretic compounds
- Docking studies were carried out for the solvated receptor structure of ATP7B protein and various diuretic compounds using docking software PyRx.
- Various diuretic compounds were docked with the receptor without any bias in order to identify the active sites.
- The interactions between the receptor and the diuretic compounds were analyzed with the help of molecular viewer.

**Table 2: Binding energy of diuretic compounds with ATP7B protein**

| S.No | Diuretic Compound | Binding Energy (Kcal / mol ) |
|---|---|---|
| 1 | Alfalfa | -2.78 |
| 2 | Ginkgo | -2.20 |
| 3 | Parsely | -2.65 |
| 4 | Cincletanine | -2.17 |
| 5 | Goldenseal | -3.09 |
| 6 | Dandelion | -1.88 |
| 7 | Thiophenalacetylene | -2.28 |
| 8 | Zigiberofficinals | -2.97 |
| 9 | Didanosine | -3.11 |
| 10 | Furosemide | -1.25 |
| 11 | Bumetamide | -0.99 |
| 12 | Ethacrynic acid | -2.57 |
| 13 | Torsemide | -2.73 |
| 14 | Chlorothiazide | -1.72 |
| 15 | Hydrochlorothiazide | -1.84 |
| 16 | Benzothiazide | -2.18 |
| 17 | Hydroflumethiazide | -1.60 |
| 18 | Acetazolamide | -1.33 |
| 19 | Spironolactone | -2.90 |
| 20 | Asteraceae | -1.87 |
| 21 | Chlorothalidone | -1.64 |
| 22 | Methyclothiazide | -1.82 |
| 23 | Bendroflumethiazide | -1.74 |
| 24 | Metalazone | -2.41 |
| 25 | Polythaizide | -1.43 |
| 26 | Quinethazone | -2.61 |
| 27 | Trichlormethazide | -1.79 |
| 28 | Aldosterone | -2.77 |
| 29 | Dichloropheamide | -2.60 |
| 30 | Etozoline | -2.59 |
| 31 | Muzolimine | -2.33 |
| 32 | Tienilic | -1.92 |
| 33 | Piretamide | -1.22 |
| 34 | Mebutizide | -1.81 |
| 35 | Clopamide | -2.68 |
| 36 | Mefruside | -2.71 |
| 37 | Clofenamide | -2.73 |
| 38 | Meticrane | -2.62 |
| 39 | Xipamide | -2.22 |
| 40 | Indapamide | -2.37 |
| 41 | Chorexolone | -2.82 |
| 42 | Fenquizone | -1.93 |
| 43 | Meralluride | -2.36 |
| 44 | Mersalyl | -3.51 |
| 45 | Theabromine | -1.99 |
| 46 | Eplerenone | -3.17 |
| 47 | Canrenone | -3.10 |
| 48 | Mozavaptan | -2.15 |
| 49 | Tolvaptan | -2.27 |

## CONCLUSION

In this study, an attempt was made to elucidate major aspects of atp7b protein and the various diuretic compounds interactions. The identification and generation of interaction for atp7b protein has unlocked a mystery that has plagued this field for years. One of the promising approaches in Wilson disease drug discovery is the development of new inhibitor for atp7b protein involved in the copper transport. The 3D structure of atp7b protein was generated and refined using MODELLER and SAVS accordingly, to study the reliability for structure based drug design. The 3D structure of atp7b protein was solvated at 310 K and energy minimized using Gromacs. Docking the atp7b protein with various diuretic compounds provided an insight into the nature of binding and interaction of ligands to the receptor. Docking studies also revealed that all the small molecule compounds are binding to the same receptor. Some differences in the residues lining extracellular region of the receptor were observed. The overall similarity of the binding pockets of these small molecule compounds and the atp7b protein offers a promising opportunity to elucidate the mode of alteration of atp7b protein. The results reported here serve to better define the parts of the

atp7b protein molecule that are essential for fighting against Wilson disease. Animal modeling studies will be carried out to design an effective chemical compound from the active principles.

## REFERENCES

[1] Ala A *et al.* (**2007**). *Lancet*. 369(9559): 397-408.
[2] Butler P *et al.* (**2001**). *Mol Genet Metab*. 110: 223–230.
[3] Eddy D.M. (**1996**). A Manual for Assessing Health Practices and Designing Practice Guidelines. Philadelphia. PA : American College of Physicians . 49: 280–286.
[4] Kitzberger R *et al*. (**2005**) . *Metab Brain Disease*. 2014: 295-302.
[5] Lim chris M *et al.* (**2006**). *Biochem biophy*. 2: 428-436.
[6] Rensing C *et al.* (**2000**). *Proc Natl Acad Sci USA 97*.110: 652–656.
[7] Sali A *et al.*(**1993**) and Blundell,T.L. *Mol. Biol*. 234:779-815.