*2012 4th International Conference on Bioinformatics and Biomedical Technology*
*IPCBEE vol.29 (2012) © (2012) IACSIT Press, Singapore*

# Comparative in- Silico inhibition study of different proteins by MIC & HCN using molecular docking.

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**Abstract.** Bhopal Gas Tragedy is one of the worst industrial catastrophes which claimed more than 2500 lives. This disaster was because of leak of methyl isocyante gas. Cyanide toxicity is one of the most contentious issues in this mass poisoning. Here an attempt has been made to analyse cyanide toxicity. Molecular docking concept is used for determination of alteration in biochemical reactions.

**Keywords:** HCN, Cyanide poisoning, Argus Lab

## 1. Introduction

On the night of 2nd/3rd December 1984, world's worst industrial accident took place at the pesticide plant in Bhopal in the state of Madhya Pradesh of India. There was a massive leak of MIC (Methyl Isocyanate) stored for a long period in the incriminated Tank 610 of the Pesticide Plant UCIL [1]. Products of pyrolysed MIC, like carbon monoxide, ammonia and hydrogen cyanide (HCN) could result in formation of carboxy-hemoglobin, methemoglobin and cyanomethemoglobin.

The aim of study was to analyze the cyanide toxicity in Bhopal Gas Disaster. In this disaster the death of victims were proposed by MIC, but the complex toxic effect of this tragedy is not because of single MIC gas. Apart from MIC, several other gases were also liberated in this disaster including HCN, carbon monoxide etc which are very lethal. Here we identified some protein targets according to symptoms of victims which are very crucial in human biochemical reaction, and checked their in-Silico inhibition.

## 2. Materials and Method

### 2.1. Preparation of Target Protein Structure

PDB is a repository of 3D structural data of bio-macromolecules (http:/www.rcsb.org/pdb).

### 2.2. Ligand Preparation

3D structure of ligand molecule MIC was retrieved from Pub Chem (CID 12228) in SDF format which was converted to pdb format by Py MOL tool.

*Identification of active site*

Active sites of proteins were identified through database catalytic site atlas of EMBL – EBI (European Bioinformatics Institute).

1. Rhodanese : - 252-THR, 251-VAL, 249-LYS, 250-GLY, 186-ARG; 248-ARG.
2. Aldolase : - 187-GLU, 33-ASP, 229-LYS.
3. Glutathione synthatase : - 305-LYS, 364-LYS, 144-GLU and 146-ASN.
4. Carbonic anhydrase :- 106-GLU and 199-THR.
5. Superoxide dismutase :- 43-HIS and 44-GLY.
6. Catalase :- 148-ASN,75-HIS,114-SER.

7. Cytochrome oxidase :-351-GLY, 376 HIS.
8. Hemoglobin
9. Cyclooxigenase

### 2.3. Molecular Docking

ArgusLab is an electronic structure program that is based on the quantum mechanics; it predicts the potential energies, molecular structures, geometry optimization of structure, vibration frequencies of co-ordinates of atoms, bond length, bond angle and reaction pathway .

## 3. Result and Discussion

Table show the binding energies obtained in ArgusLab for complexes of the receptors and pyrolized product of MIC i.e. HCN. More negative binding energies correspond to higher binding affinities. H – BOND (LIGAND WITH RECEPTOR) in table shows amino acids are active site residues of protein which were taking part in formation of hydrogen bond with ligand molecules.

TABLE: Docking Results by Argus lab

| S. No | RECEPTOR | PDB ID | H – BOND (LIGAND WITH RECEPTOR) | E-VALUE (kcal/mol) | LIGAND | INHIBITING MOLECULE (LIGAND) |
|---|---|---|---|---|---|---|
| 1 | ALDOLASE | 4ALD | 33-ASP | -5.18 | HCN | **HCN** |
| | | | 338-CYS | -2.44 | MIC | |
| 2 | CYTOCHROME OXIDASE | 1OCO | 3574-HEM | -4.77 | HCN | **HCN** |
| | | | 316-THR | -4.46 | MIC | |
| 3 | RHODANESE | 2ORA | 186-ARG<br>248-ARG | -4.89 | SCN | **SCN** |
| | | | 116-ARG | -4.57 | MIC | |
| 4 | 3MARCPTOPYRUVATE SULPHUR TRANSFERASE | 3OLH | 65-SER | -4.25 | MIC | **NO INHIBITION** |
| 5 | CARBONIC ANHYDRASE | 12CA | 106-GLU<br>119-HIS<br>117-GLU<br>107-HIS | -5.41 | HCN | **HCN** |
| | | | 200-THR | -4.5 | MIC | |
| 6 | CATALASE | 1DGB | 148-ASN<br>75-HIS | -4.92 | HCN | **HCN** |
| | | | NO HYDROGEN BOND | -4.06 | MIC | |
| 7 | SUPEROXIDE DISMUTASE | 1HL5 | 43-HIS<br>44-GLY | -4.02 | HCN | **HCN** |
| | | | NO HYDROGEN BOND | -3.82 | MIC | |
| 8 | HEMOGLOBIN | 3DUT | 573-HEM | -4.52 | HCN | **HCN** |
| | | | 490-VAL | -4.48 | MIC | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | CYCLOOXIGENASE | 1PRH | 1141-HEM 203-GLN | -4.72 | HCN | **HCN (ACTIVATING)** |
| | | | NO HYDROGEN BOND | -4.45 | MIC | |
| 10 | GLUTATHIONE SYNTHATASE | 2HGS | 144-GLU 145-ILE | -5.29 | HCN | **HCN** |
| | | | 145-ILE | -4.57 | MIC | |

Cyanide is inhibiting cytochrome oxidase [2] terminal enzyme of ETC which finally delivers electron to molecular O2 and reduces it to water final product of oxidative phosphorylation and produces energy in form of ATP.

As a result of mitochondrial electron transport inhibition by cyanide, excess reactive oxygen species (ROS) are generated at complexes I and III, producing intense oxidative stress and inhibition of enzymes catalase, glutathione peroxidase, and superoxide dismutase by cyanide can lead to excessive lipid peroxidation of neuronal cell [3] may be the reason of psychological and neurological disorders in victims. Cyanide is also binding with hemoglobin and forms cyanhemoglobin which makes O2 unable to bind hemoglobin and result in histotoxic hypoxia condition. Cyclooxigenase activation by cyanide produce peroxides (result of oxidative stress) can lead to production of prostaglandins which are mediators of inflammation reaction in the body may be the reason of observed inflammation in victims. Inhibition of cyanide detoxification enzyme rhodanese seems had played pivotal role in this mass poisoning as docking result shows SCN- inhibits rhodanese which is its own product and literature also suggest that product inhibition of rhodanese may take place when cyanide concentration approaches to optimum level. As docking result shows glutathione synthatase is also inhibited by cyanide which is key enzyme in important antioxidant glutathione biosynthesis. This inhibition will result in reduced level glutathione as seen in victims (in autopsy report of victims reduced content of cellular glutathione was reported).

HCN is detoxified to thiocyanate (SCN-) by the mitochondrial enzyme rhodanese; rhodanese catalyzes the transfer of sulfur from thiosulfate to cyanide to yield thiocyanate, which is relatively nontoxic[4]. The enzyme rhodanese is present to a large excess in the human body relative to its substrates [5]. This enzyme demonstrates zero-order kinetics, and the limiting factor in the detoxification of HCN is thiosulphate. However, other sulfur-containing substrates, such as cystine and cysteine, can also serve as sulfur donors. Other enzymes, such as 3-mercapto-pyruvate sulfur transferase, can convert cyanide to thiocyanate ATSDR (2006). Thiocyanate is eliminated in the urine. But the docking result shows that rhodanese is inhibited by HCN where as HCN has no effect on 3-mercapto-pyruvate sulfur transferase Table.

Glutathione helps process medications and cancer-causing compounds (carcinogens), and helps build DNA, proteins, and other important cellular components. Diseases associated with altered glutathione metabolism; Defects in enzymes of the c-glutamyl cycle, Parkinson's disease, Cystic fibrosis, Liver disease, and Liver disease.

## 4. Conclusion

Bhopal gas disaster is one of the worst industrial disasters, caused by the massive leak of 42 tons of stored MIC. In this mass poisoning more than 2500 people died. This disaster cannot be attributed by the exposure of a single chemical like MIC. Pyrolysis of MIC produces more lethal gases including HCN. This HCN seems to be the main culprit of acute phase mortality. Many long term clinical studies had been undertaken for evaluation of Health effect of MIC but not about HCN. These long term effects of HCN must have to be studied.

## 5. References

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