**RESEARCH ARTICLE**

**In silico docking of methyl isocyanate (MIC) and its hydrolytic product (1,3-dimethylurea) shows significant interaction with DNA Methyltransferase 1 suggests cancer risk in Bhopal-Gas-Tragedy survivors**

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**Abstract**

DNA methyltransferase 1 (DNMT1) is a relatively large protein family responsible for maintenance of normal methylation, cell growth and survival in mammals. Toxic industrial chemical exposure associated methylation misregulation has been shown to have epigenetic influence. Such misregulation could effectively contribute to cancer development and progression. Methyl isocyanate (MIC) is a noxious industrial chemical used extensively in the production of carbamate pesticides. We here applied an *in silico* molecular docking approach to study the interaction of MIC with diverse domains of DNMT1, to predict cancer risk in the Bhopal population exposed to MIC during 1984. For the first time, we investigated the interaction of MIC and its hydrolytic product (1,3-dimethylurea) with DNMT1 interacting (such as DMAP1, RF1TS, and CXXC) and catalytic (SAM, SAH, and Sinefungin) domains using computer simulations. The results of the present study showed a potential interaction of MIC and 1,3-dimethylurea with these domains. Obviously, strong binding of MIC with DNMT1 interrupting normal methylation will lead to epigenetic alterations in the exposed humans. We suggest therefore that the MIC-exposed individuals surviving after 1984 disaster have excess risk of cancer, which can be attributed to alterations in their epigenome. Our findings will help in better understanding the underlying epigenetic mechanisms in humans exposed to MIC.

**Keywords:** Molecular docking - *in silico* interaction - methyl isocyanate (MIC) - 1,3-dimethylurea - DNMT1

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**Introduction**

There is increasing evidence that exposure to toxic chemicals primarily affect the epigenome leading to the alteration of normal epigenetic process (Hou et al., 2011). In humans, chemically-induced epigenetic alterations has been reported to play an important role in cancer development and progression (Fraga et al., 2005; Baccarelli and Bollati, 2009; Shrivastava et al., 2013). Methyl isocyanate (MIC) is a toxic chemical used as an intermediate in the production of carbamate pesticides (HSFS, 2002; Senthilkumar et al., 2011; 2012; 2013). Accidental leakage of MIC from storage tank number E610 of Bhopal Union Carbide India Limited (UCIL) pesticide production plant on 3rd December, 1984 resulted in the mortality of 3600 inhabitants and about 2,00,000 were severely exposed. This catastrophe was caused by the introduction of water with 40 tonnes of MIC. Analysis conducted after Bhopal episode have concluded the presence of several toxic chemicals residues, including 1,3-dimethylurea in E610. MIC reacts exothermically with water to form carbon dioxide, methylamine, dimethylurea and/or trimethylbiuret (Stringer et al., 2002; NOAAS, 2015).

Exposure to MIC causes several respiratory disorders and chronic obstructive pulmonary disease (COPD) (Vandenplas et al., 1993; Baur et al., 1994; De, 2012; 2013). Besides, the immunotoxic (Karol et al., 1987), genotoxic (Conner et al., 1987; Tice et al., 1987), reproductive and developmental toxic effects (Schwetz et al., 1987) are well-documented in humans and animals. However, the etiology of MIC in carcinogenesis is far from completely understood (Senthilkumar et al., 2012; Senthilkumar, 2012). Data from animal studies have shown that the carcinogenic potency of MIC is low and weak (Gassert et al., 1986; Ennever and Rosenkranz, 1987; Bucher and Uraih, 1989). Evidence from *in vitro* studies suggest MIC-induced oncogenic transformation
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DNA methylation is essential for the maintenance of normal epigenetic process (Miller et al., 1974). Aberrant methylation is the major cause of several cancers, shows both hyper- and hypo-methylation patterns (Robertson, 2005). Hypermethylation of tumor suppressor genes in human cells (Mishra et al., 2009abc; Raghuram et al., 2010; Hariom and Mishra, 2011; Hariom et al., 2011). But still, toxic chemical substances analyzing agencies are categorizing MIC as a non-carcinogenic substance (Senthilkumar et al., 2012).

Almost three decades elapsed of Bhopal disaster, only a few efforts have been made to understand the cancer risk in the MIC-exposed survivors (Malla et al., 2011; Senthilkumar et al., 2011; 2012; 2015). Emerging epidemiological reports from Bhopal, India suggest diverse cancer pattern amongst the MIC-gas exposed survivors (Dikshit and Kanhere, 1999; Ganesh et al., 2005; Senthilkumar et al., 2011). It was therefore, worthwhile to investigate the underlying mechanisms of MIC on epigenetic alterations/DNA methylation using computational methods. In the present study, we aimed to understand the in silico interaction of MIC and its hydrolytic product (1,3-dimethylurea) with DNA methyltransferase (DNMT) domains, in order to reveal the cause for cancer risk in Bhopal population exposed to MIC during 1984.

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Table 1. Detailed Interaction Profiles of MIC and 1,3-Diurea with DNA binding CXXC (1 - 3), DMAP1, RFTS Region (1 and 2), catalytic SAH/SAM and Sinefungin site

| Ligand                                      | Docking Score | XP Score | Glide Score | Glide E-Model | H- Bonding | Length of H Bond (Å) | Acceptor Angle | Donor Angle | Bonded Chain  |
|---------------------------------------------|---------------|----------|-------------|---------------|------------|---------------------|----------------|-------------|---------------|
| DNA Binding CXXC Region 1                   |               |          |             |               |            |                     |                |             |               |
| 1,3-Dimethylurea                            | -2.738        | -2.738   | -2.738      | -20.394       | Glycine (684) | 2.18(H)             | 151.653        | 121.953     | Backbone      |
| MIC                                         | -1.852        | -1.852   | -1.852      | -9.714        | Lysine (683)  | 1.96(O)             | -              | 133.694     | Backbone      |
| DNA Binding CXXC Region 2                   |               |          |             |               |            |                     |                |             |               |
| 1,3-Dimethylurea                            | -2.237        | -2.237   | -2.237      | -15.402       | Cystine (686) | 2.21(H)             | 157.022        | 90.87       | Backbone      |
| MIC                                         | -1.223        | -1.223   | -1.223      | -8.239        | Arginine (690) | 2.30(O)             | -              | 98.767      | Side Chain    |
| DNA Binding CXXC Region 3                   |               |          |             |               |            |                     |                |             |               |
| 1,3-Dimethylurea                            | -2.808        | -2.808   | -2.808      | -17.272       | Methionine (696) | 2.05(O)             | -              | 157.33      | Backbone      |
| MIC                                         | -2.02         | -2.02    | -2.02       | -9.414        | Glutamic Acid (698) | 1.86(H)             | 160.345        | 155.454     | Side Chain    |
| DNMT1 Associated Protein 1                  |               |          |             |               |            |                     |                |             |               |
| 1,3-Dimethylurea                            | -2.205        | -2.205   | -2.205      | -14.916       | Asparagine (1379) | 2.03(H)             | 152.599        | 140.665     | Backbone      |
| MIC                                         | -1.752        | -1.752   | -1.752      | -9.335        | Aspartic Acid (1416) | 2.07(O)             | -              | 102.877     | Side Chain    |
| Replication Focii Targeting Sequence Region 1|               |          |             |               |            |                     |                |             |               |
| 1,3-Dimethylurea                            | -2.977        | -2.977   | -2.977      | -16.621       | Phenylalanine(483) | 2.37(H)             | 143.35         | 121.327     | Backbone      |
| MIC                                         | -1.783        | -1.783   | -1.783      | -6.432        | Proline (403)  | 1.72(H)             | 166.236        | 124.141     | Backbone      |
| Replication Focii Targeting Sequence Region 2|               |          |             |               |            |                     |                |             |               |
| 1,3-Dimethylurea                            | -2.702        | -2.702   | -2.702      | -19.087       | Threonine (467) | 2.33(N)             | -              | 98.906      | Side Chain    |
| MIC                                         | -1.772        | -1.772   | -1.772      | -9.422        | Glycine (468)  | 2.03(O)             | -              | 156.032     | Backbone      |
| Catalytic Methytransferase Domain (SAH/SAM) |               |          |             |               |            |                     |                |             |               |
| 1,3-Dimethylurea                            | -3.182        | -3.182   | -3.182      | -27.699       | Valine (1580)  | 2.07(O)             | -              | 144.91      | Backbone      |
| MIC                                         | -2.729        | -2.729   | -2.729      | -13.559       | Leucine (1151) | 2.17(O)             | -              | 111.548     | Backbone      |
| Catalytic Methytransferase Domain (Inhibitor-Sinefungin)| | | | | Valine (1576)  | 1.73(O)             | -              | 128.092     | Backbone      |
| 1,3-Dimethylurea                            | -3.086        | -3.086   | -3.086      | -28.544       | Leucine (1151) | 2.24(O)             | -              | 102.162     | Backbone      |
| MIC                                         | -2.286        | -2.286   | -2.286      | -10.899       | Serine (1146)  | 2.30(H)             | 125.269        | 112.212     | Backbone      |
| DNA methylation is essential for the maintenance of normal epigenetic process (Miller et al., 1974). Aberrant methylation is the major cause of several cancers, shows both hyper- and hypo-methylation patterns (Robertson, 2005). Hypermethylation of tumor suppressor genes

Figure 1. DNMT1 and its Diverse Interacting Domains
Protein Preparation Wizard (PPW). These raw crystal the resolution range from 1.45 to 3.60 Å. The raw crystal (4IEJ, 3EPZ, 3PTA, 3SWR) by X-Ray diffraction with retrieved from the Protein Data Bank (PDB, 2014a-d) (ID: RFTS, DNMT1 in complex with DNA, and Sinefungin) domains of DNMT1 using MIC and 1, 3-Dimethylurea as ligands. We used Schrödinger GLIDE Selection and preparation of protein.

Materials and Methods

Source, selection and preparation of protein

A total of four human DNMT1 proteins (viz. DMAP1, RFTS, DNMT1 in complex with DNA, and Sinefungin) and their three-dimensional (3-D) crystal structures were retrieved from the Protein Data Bank (PDB, 2014a-d) (ID: 4IEJ, 3EPZ, 3PTA, 3SWR) by X-Ray diffraction with the resolution range from 1.45 to 3.60 Å. The raw crystal structures were subjected to docking preparation using Protein Preparation Wizard (PPW). These raw crystal structures were scrutinized for excessive heavy atoms, improper bonding, missing of side chains, presence of co-crystallized ligand, water molecules, metal ions, and cofactors etc. Using PPW, hydrogen and appropriate bonds were added to the structures. Metals were treated by breaking bonds and assigned with formal charges. Disulfide bonds were added between two adjacent sulphur molecules. Overlaps were removed, water molecules were deleted, and missing loops were filled using the PRIME. N-Acetyl (ACE) and N-Methyl Amide (NMA) groups were added to uncap the N- and C-terminus. In addition, the hetro-groups were also detached. Protonation and metal charges states were generated. Hydrogen bonds were optimized using the PROT ASSIGN. Finally, the protein structure was subjected to restrained minimization in the Impref utility using the OPLS2005 force field. (Jacobson et al., 2004; Shelley et al., 2007; Shivakumar et al., 2010; Sastry et al., 2013).

Preparation of ligand

3-D ligand structures of MIC and 1,3-dimethylurea were retrieved from ZINC Database (Irwin and Shoichet, 2005). Ligand geometry was optimized by generating the structural variants, which reduced the structural problems in ligands and these structures were checked through LIGPREF. Ionization state was generated in the default pH 7.0±2.0 using ionizer. The structure was desalted to retain molecule with the largest number of atoms. Tautomers were generated for each neutralized/ionized molecule. Finally, around 32 stereoisomers were created with retained specific chiralities according to Greenwood et al. (2010).

Generation of receptor grid

The receptor grid is a 3-D boundary for ligand binding. The grid was generated specifically for various domains of the proteins. The residues of DMAP1 interacting with DNMT1 was determined using the structure (PDB ID: 4IEJ). The grid was created on the centroid region of selected residues in which DMAP1 and DNMT1 interact, in order to confine the ligands to that specific region during the docking process. Similarly, CXXC region interacting with DNA was analyzed using the structure (PDB ID: 3PTA) and grids were generated separately in three regions. RFTS Domain (PDB ID: 3EPZ) was also analyzed using the same procedure and the grid was generated for two different regions of DNMT1, where the interaction with replication foci occurs. With reference to catalytic domain of DNMT1, SAH is previously co-crystallized with the structure (PDB ID: 3PTA) and therefore the grid generation process was totally different. The grid was generated at the centroid of selected ligand with co-crystallized SAH. The grid was generated around this ligand and grid file also lack this ligand. Typically, using this procedure the grid was generated and ligands were docked only for this region. Similar procedure was adapted for Sinefungin (PDB ID: 3SWR). Van Der Waals scaling was made for all the grids using default scaling factor of 1.0 and partial cut-off charges were 0.25 as described earlier (Friesner et al., 2004).
Figure 2. Docked Position of CXXC Domain (A - Region 1, B - Region 2, and C - Region 3), DMAP1 (D), RFTS (E-Region 1 and F - Region 2), SAH/SAM (G), and Sinefungin Binding Domain (H) of DNMT1 with Ligands. Structural view of hydrogen bond (green dashed line) showing the distance, where as the ligand interaction (solid pink arrows) represents electrostatic relations (H-Bond) with main backbone of protein and side chain (dashed pink line).
**In silico docking**

The docking sites for ligand were presented by grids. During the docking procedure of Schrodinger GLIDE, the ligands and grids were assessed and then subjected to induced fit flexible docking using Extra Precision (XP) mode. While docking the protein, the receptor was rigid, but the ligand was flexible. Van Der Waals radii scaling were done with the default scaling factor 0.8 and a partial charge cut-off as 0.15. Different conformations were created internally by GLIDE and subjected through a set of filters, viz. Euler angles, grid based force field evaluation and refinement, and Monte Carlo energy minimization according to Friesner et al. (2006). Finally, the docked conformers were estimated using GLIDE score and a single best position per ligand was generated as an output as described earlier (Halgren et al., 2004). For each ligand and protein site, the docked positions were determined accordingly to the docking profile (such as docking, XP GLIDE, and standard precision GLIDE scores). In addition, GLIDE-E model was calculated and it is directly proportional to the elevated negative value i.e. increased negative value is considered as a better docking score. This score is the measure of strength and stability of the bound-ligand-protein.

**Results**

Table 1 depicts the docking profile of MIC and 1,3-dimethylurea with DNMT1 (Figure 2A-H). Both the ligands showed significant interaction with amino acids and stable hydrogen bond formation with core- and side-chains on various regions of DNMT1. Hydrogen bonds formed by ligands with the protein residue were found between 1.5 to 2.5 Å. The minimum donor and acceptor angle is 120° and 90°, respectively. 1,3-dimethylurea has shown a strong interaction as compared to MIC. This interaction is due to the size and complexity of the molecule. Besides, the strongest binding was observed for SAH and Sinefungin region (Figure 2 G and H).

**Discussion and Conclusion**

**In silico docking**

Interaction of Methyl Isocyanate and 1, 3-Diurea with Diverse Domains of DNMT1: a Molecular Docking Approach

In agreement to our results, several studies proved that the global hypomethylation is due to many environmental and agricultural chemicals (Hou et al., 2011) have plausible role in cancer etiology. Interestingly, recent docking studies conducted by Shrivastava et al. (2010; 2013) and Tripathi et al. (2015) also demonstrated a comprehensive understanding of MIC with different immunoproteins against Tuberculosis. To support our findings, recent epidemiological and experimental studies also indicate genetic instability in the MIC-exposed...
survivors, are highly susceptible to cancers (Malla et al., 2011; Senthilkumar et al., 2011; 2013; 2015). Almost 30 years elapsed after Bhopal disaster, owing to several bias and factors it is difficult to understand the direct or indirect effects of MIC in the exposed survivors. Hence, this kind of in silico docking analysis will be helpful to elucidate the underlying disease mechanism in the MIC-exposed survivors and their epigenetic status. To our knowledge, this is the first study to report the interaction of MIC and 1,3-dimethylurea with DNMT1 (DMAP1, RFTS, and CXXC) and catalytic (SAM/SAH and Sinefungin) domains.

In summary, we found a potential interaction of MIC and 1,3-dimethylurea with these domains. Apparently, the stronger binding of MIC with DNMT1 interrupts the normal methylation will lead to epigenetic alterations in the exposed survivors. We conclude therefore that the MIC-exposed individuals surviving after 1984 disaster have excess risk of cancer, which can be attributed to alterations in their epigenome. Our findings will help in better understanding the underlying epigenetic mechanisms in humans exposed to MIC.

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