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# Comparative Prediction of binding site of Organophosphorus, Carbamate and Synthetic pyrethroid Pesticides on Human Cyclin-dependent Protein kinases Cdk2 and Cdk4

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## ABSTRACT

The pesticides are used for plant protection and control pests. They enter into the cell cycle thereby affecting important regulatory elements. The impact of organophosphorus, carbamate and synthetic pyrethroid pesticides on cyclin-dependent protein kinases Cdk2 and Cdk4 in human being have been evaluated in the present study. The binding affinity of selected pesticides with human Cdk2 and Cdk4 [using Molegro Virtual Docker (MVD)] are in decreasing order i.e. organophosphorus > carbamate > Synthetic pyrethroid. Many of these alterations affect the cyclin-dependent kinases (CDKs) and their regulation interfere in the cell cycle and cause irregular cell division which may lead to carcinogenesis in human beings.

**Keywords:** Organophosphorus, Carbamate and Synthetic pyrethroid, Pesticides, Cyclin- dependent kinases, Molegro Virtual Docker.

## 1. Introduction

The pesticides are used to control pest around the world to manage pest problems. They widely affect human health including short-term impacts such as headaches and nausea and long term impacts such as cancer, reproductive harm and endocrine disruption [1]. DDT can enter into the cell cycle by directly affecting key regulatory elements and activate breast cancer cells [2], 1, 2-dibromoethane and folpet pesticides alter normal cell cycle and promote chromosomal abnormalities and their tolerance and cause neoplastic transformation which is responsible for tumor progression [3]. Parathion are able to alter genes which are involved in the regulation of cell cycle [4] while, Kannan [5] and Saleh [6] reported that 10-200 micro M endosulfan and 1-10 nM paraoxon respectively causes apoptotic cell death in a leukemia cell line by disruption of mitochondria.

The cell cycle machinery is frequently altered in cancer affecting cyclin-dependent protein kinases (Cdks) and their regulation including initiation of DNA replication, mitosis and prevention of re-replication. The sequential activation and inactivation of cyclin-dependent protein kinases (Cdks) regulate mammalian cell cycle. CDK activities are controlled by several different mechanisms, including binding of positively acting regulatory cyclin subunits, inhibition by CDK inhibitors, phosphorylation of CDKs by CDK activating kinases and dephosphorylation by cell-cycle regulated phosphatases [7, 8]. The Cyclin/CDK complexes control cell proliferation by phosphorylating key regulatory proteins at specific transition points of the cell cycle [9].

In the present study it has been observed that binding of some pesticides such as organophosphorus (sarin), carbamate (methyl carbamate) and synthetic pyrethroid (cypermethrin and deltamethrin) effect on cyclin-dependent protein kinase Cdk2 and Cdk4. They were docked with sarin, methyl carbamate, cypermethrin, deltamethrin and observed the comparative Mole Dock Score, Root-mean square deviation (RMSD), binding affinity, interacting residues of human Cdk2 and Cdk4, number of H-bond interaction, docking score and interacting interaction of residues using MVD. The motivation behind this was to investigate binding site of pesticides on cyclin-dependent protein kinases.

## 2. Materials and Methods

Three dimensional X-ray crystallized structure of human cyclin-dependent protein kinase 2 (Cdk2) (PDB: 4EOI) was downloaded from the protein data bank [10]. Cdk2 promotes DNA replication and is a promising cancer therapeutic target. The protein was taken as receptor
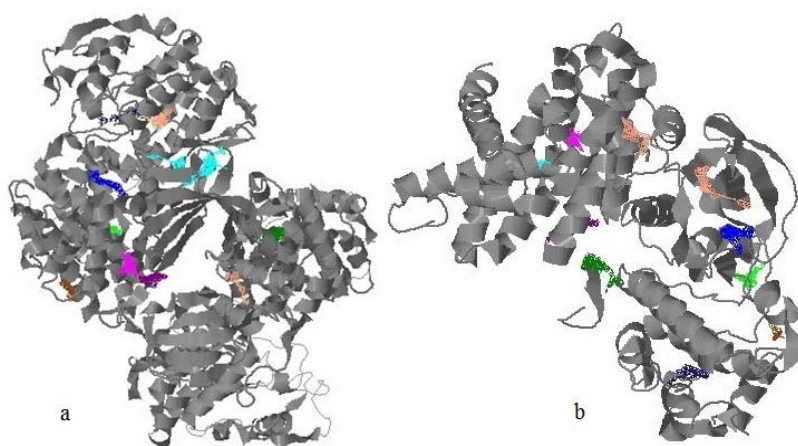
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protein and most suitable site was predicted by using q site finder ligand binding site prediction (http://www.modelling.leeds.ac.uk). Three dimensional X-ray crystallized structure of human cyclin-dependent kinase 4 (Cdk4) (PDB: 2W96) was downloaded from the protein data bank [11]. The downloaded Cdk4 complex is a key regulator of the transition through the G1 phase of the cell cycle and is frequently activated by somatic genetic alterations in multiple tumor types.

Cdk2 and Cdk4 have been selected for docking with ligand Organophosphorus [sarin (CID_7871)], carbamate [methyl carbamate (CID_11722)] and Synthetic pyrethroid [cypermethrin (CID_2912) & deltamethrin (CID_40585)] pesticides. The selected ligands (pesticides) were downloaded from Pub Chem Compound (http://www.ncbi.nlm.nih.gov). Docking study was done with Molegro Virtual Docker (MVD). It is automated docking software with fast processing. The binding site cavity detection was performed by q site finder ligand binding site prediction tool (Fig. 1).



**Fig 1:** Binding site for Cyclin-dependent protein kinases Cdk2 (a) and Cdk4 (b)

The docking simulation was performed by using docking software, namely MVD for the selected pesticides (ligands) and Cdk2 and Cdk4. It shows mole dock score, root-mean-square deviation (RMSD), affinity (the estimated binding affinity in kj /mol), docking score and interacting interaction (the interaction energy among the pose and the cofactor), number of H-bond and interaction between interacting residues of receptor human cyclin-dependent protein kinase Cdk2 and Cdk4 [12]. MVD visualizer is used for interaction site prediction.

## 3. Results and Discussion

The comparative results obtained (using MVD) from docking simulation with Cdk2 and Cdk4 are given in Table-1. The interaction analysis for binding of human Cdk2 and Cdk4 with Organophosphorus (sarin), Carbamate (methyl carbamate) and Synthetic pyrethroid (cypermethrin and deltamethrin) pesticides was done to find out the residues that are involved in binding site residues and number of hydrogen bonds are involved in interaction among selected pesticides, Table 2. The energy bound conformation with lower value of selected ligands and pesticides show hydrogen bond interactions which are given in Figures 2 and 3. Docking energy for most of the pesticides was found favorable for Cdk2 and Cdk4 which shows that these compounds can get stuck due to positive interaction [13].

**Table 1:** Comparative Docking Simulation Results of Pesticides with Human Cdk2 and Cdk4 using MVD.

| | Ligands (Pesticides) | Cdks | MoleDock Score | RMSD | Affinity | Intracting | Docking Score |
|---|---|---|---|---|---|---|---|
| 1. | Sarin (Organophosphorus) | Cdk2 | -56.1239 | 0.164314 | -24.8344 | -57.4388 | -57.5918 |
| | | Cdk4 | -35.7989 | 3.73165 | -23.377 | -36.9859 | -39.6291 |
| 2. | Methyl carbamate (Carbamate) | Cdk2 | -41.1317 | 0.286771 | -36.0997 | -43.3987 | -43.1612 |
| | | Cdk4 | -38.8495 | 0.154271 | -25.0763 | -41.03 | -42.4332 |
| 3. | Cypermethrin (Synthetic pyrethroid) | Cdk2 | -81.8699 | 10.4489 | -37.2476 | -84.0544 | -81.5399 |
| | | Cdk4 | -80.562 | 3.83371 | -36.3174 | -75.0172 | -79.5908 |
| | Deltamethrin (Synthetic pyrethroid) | Cdk2 | -93.8633 | 4.30564 | -23.814 | -112.668 | -93.6867 |
| | | Cdk4 | -135.168 | 5.77634 | -36.8851 | -156.378 | -134.86 |

The hydrogen bonds are significant for interaction of biomolecules. The Organophosphorus pesticide (sarin) forms low binding energy complex as compared to methyl carbamate with Cdk2 which shows binding affinity with Lys33, Asp145, Ala144 and forms three hydrogen bonds with Lys33, Asp145 (Table -2 and Fig. 2 a). The carbamate (methyl carbamate) shows very high binding energy to
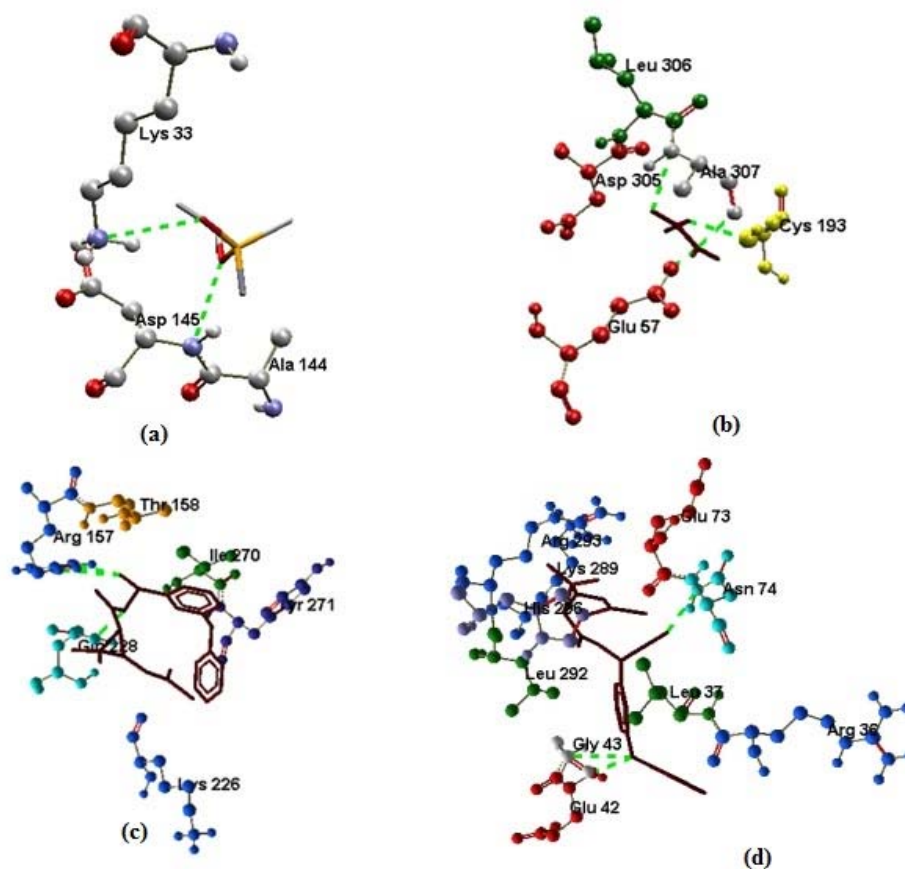
bind with human Cdk2 and it interacts with Leu306, Asp305, Ala307, Cys193, Glu57 residues of human Cdk2. All these residues involved in binding belong to the cavity-1 and forms 4 hydrogen bonds with Ala307, Cys193, Glu57 (Table -2 and Fig. 2 b). The Synthetic pyrethroid (Cypermethrin) forms low binding energy complex as compared to sarin and shows binding affinity with Gln228, Arg157, Ile270, Thr158, Lys226, Tyr271 which forms three hydrogen bonds with Gln228, Arg157 residues of human Cdk2 (Table -2 and Fig.- 2 c). The Synthetic pyrethroid (deltamethrin) forms low binding energy complex as compare to

cypermethrin, it shows binding affinity with Glu73, Asn 74, Glu42, Arg36, Leu 37, Leu292, His296, Arg293, Lys289 residues of human Cdk2 Whereas, deltamethrin formed three hydrogen bonds with Glu73, Asn 74, Glu42 (Table 2 and Fig. 2d).

The binding affinity of selected pesticides methyl carbamate, cypermethrin, deltamethrin and sarin at the active site of human Cdk2 using MVD was obtained in decreasing order i.e. organophosphorus > carbamate > Synthetic pyrethroid ( sarin > methyl carbamate > cypermethrin > deltamethrin).

**Table 2:** Human Cdk2 and Cdk4 residues interact with Pesticides using MVD (highlighted residues are involved in H-bonding interaction with ligands).
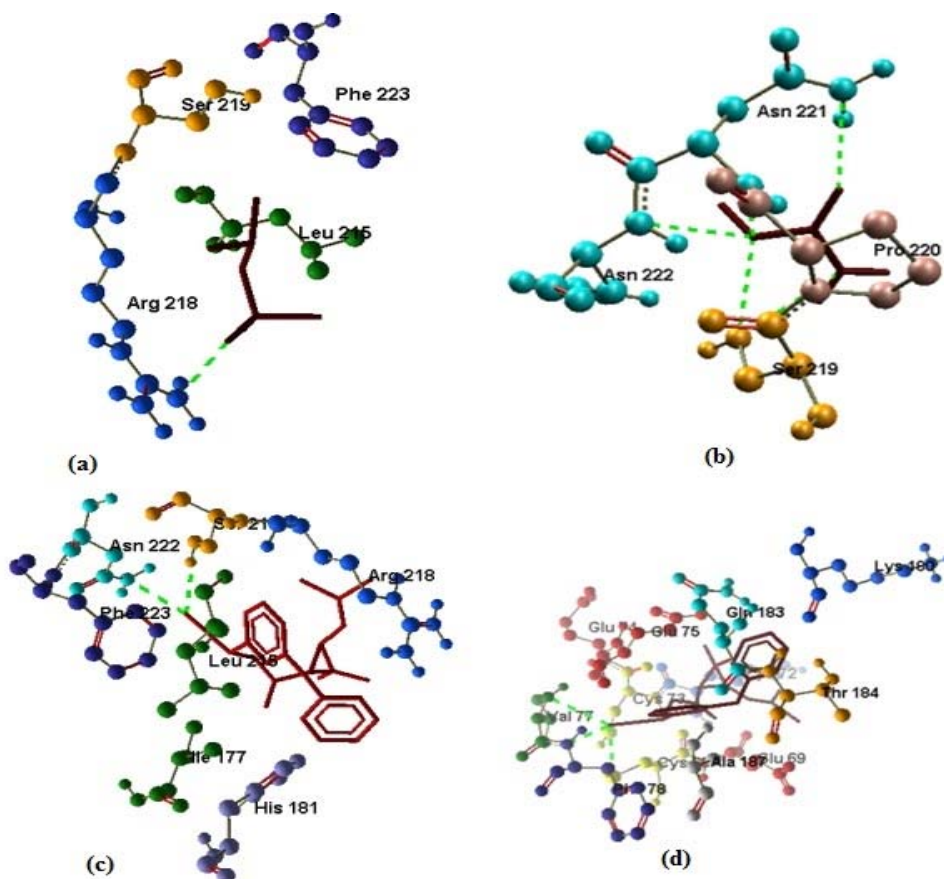
| S. No. | Ligands (Pesticides) | Cdks | Interacting residues of receptor Cdk2 and Cdk4 | No. of H-bond interaction |
|---|---|---|---|---|
| 1. | Sarin (Organophosphorus) | Cdk2 | **Lys33**, Asp145, Ala144 | 02 |
| | | Cdk4 | **Arg218**, Ser 219, Phe223, Leu215 | 01 |
| 2. | Methyl carbamate (Carbamate) | Cdk2 | **Ala307**, **Cys193**, **Glu57**, Leu306, Asp305 | 04 |
| | | Cdk4 | **Asn 221**, **Ser 219**, **Asn222**, Pro220 | 05 |
| 3. | Cypermethrin (Synthetic pyrethroid) | Cdk2 | **Gln228, Arg157**,Ile270, Thr158, Lys226,Tyr271 | 03 |
| | | Cdk4 | **Ser 219**, **Asn22**, Ile 177, His181, Arg 218, Phe223, Asn 22 | 02 |
| | Deltamethrin (Synthetic pyrethroid) | Cdk2 | **Glu73**, **Asn 74**, **Glu42**, Arg36, Leu3, Leu292, His296, Arg293,Lys289 | 03 |
| | | Cdk4 | **Val77, Cys68**, **Phe78**, Glu183, Lys180, Thr184, Ala187, Glu69**,** Glu74, Glu75, Lys72, Cys73 | 03 |



**Fig 2:** Docked conformation of hydrogen bonding interaction view of (a) Organophosphorus (sarin), (b) Carbamate (methyl carbamate) and Synthetic pyrethroid cypermethrin (c) & deltamethrin (d) Pesticides with interacting Cyclin-dependent kinases (Cdk2) at the active site cavity.

The Organophosphorus pesticide (sarin) forms very high binding energy complex with Cdk4. It shows binding affinity with Arg218, Ser 219, Phe223, Leu215 which forms one hydrogen bond with Arg 218 (Table 2 and Fig. 3a), methyl carbamate shows low binding energy complex as compare to sarin to bind with human Cdk4 and it interacts with Asn 221, Ser 219, Asn222, Pro220 residues of human Cdk4. All these residues involved in binding belong to the cavity-1. The methyl carbamate forms five hydrogen bonds with Asn 221, Ser 219, Asn 222 (Table 2 and Fig. 3b). The hydrogen bonding is very significant for interaction of biomolecules. The cypermethrin forms low binding energy complex as compare to methyl carbamate shows binding affinity .

with Ser 219, Asn22, Ile 177, His181, Arg 218, Phe223, Asn 22 and forms two hydrogen bonds with Ser 219, Asn22 residues of human Cdk4 (Table 2 and Fig. 3c). The deltamethrin forms low binding energy complex as compare to cypermethrin, it shows binding affinity with Val77, Cys68, Phe78, Glu183, Lys180, Thr184, Ala187, Glu69, Glu74, Glu75, Lys72, Cys73 residues of human Cdk 4. Whereas, deltamethrin formed three hydrogen bonds with Val77, Cys68, Phe78 (Table 2 and Fig. 3d). The binding affinity of selected pesticides methyl carbamate, cypermethrin, deltamethrin and sarin at the active site of human Cdk4 using MVD is in decreasing order i.e. sarin > methyl carbamate > cypermethrin > deltamethrin



**Fig 3:** Docked conformation of hydrogen bonding interaction view of (a) Organophosphorus (sarin), (b) Carbamate (methyl carbamate) and Synthetic pyrethroid cypermethrin (c) & deltamethrin (d) Pesticides with interacting Cyclin-dependent kinases (Cdk4) at the active site cavity.

It is well known that pesticides (organophosphate, carbamate and synthetic pyrethroid) cause cancer as well as tumor in animals [14]. Components of the cell cycle machinery are frequently altered in cancer. Many of these alterations affect the cyclin-dependent kinases (CDKs) and their regulation. We have evaluated the binding of pesticide on human cyclin-dependent kinases (Cdk2 and Cdk4). Cyclin-dependent kinases (CDKs) such as Cdk2 and Cdk4 play crucial role in the cell cycle transitions [15-18]. It has been identified that deregulation of the cyclin D /Cdk4 pathway induce multiple tumor and altered cell proliferation in human beings [19-25].

## 4. Conclusion
The unlimited exposure of pesticides is one of the main reasons to develop carcinogenic cells in human. Methyl carbamate, cypermethrin, deltamethrin and sarin, pesticides interact with human Cdk2 and Cdk4 and make hydrogen bonds which disturb cell cycle and initiate irregular cell division. Cell-cycle

dysregulation delays activity of the CDK1/cyclin B complex in cell cycle, activate tumor cells and initiates human cancers. Since cancers develops in years or decades after the primary dysfunction of a single cell and therefore it is of great importance to develop prevention strategies on the basis of the knowledge of the undesirable molecular target such as pesticides.

## 5. Acknowledgement

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