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The Effects of Formalin (Formyl Tetrahydrofolate) on CDK1 Protein on the Oocyte Maturation Process: Quantitative Structure Toxicity Relaionships (QSAR) In Silico Analysis

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ABSTRACT

Formalin is an aqueous solution of formaldehyde. Formalin can trigger the formation of ROS causing oxidative stress, and has an impact on changes in the CDK1 protein which has a role in oocyte maturation. The researchers were able to predict in silico mutagenicity and formyl tetrahydrofolate toxicity to CDK1 protein in oocyte cell maturation using the Molegro Virtual Docker computer program and the comparison compound fipronil. The receptor CDK1/CyclinB1/CKS2, code PDB: 4Y72, was used with the LZ9 ligand. Using the pkCSM online tool program, investigate in silico qualitative structure activity relationships (QSAR) to predict pharmacokinetic properties (ADME), toxicity of potential medicinal compounds, environmental contaminants, and poisons. The bond energy resulting from docking between formalin, LZ9 ligand, and fipronil comparators at the target receptor was compared for data analysis. The more stable the bonds are, the lower the bond energy of the ligands to the target receptor. The bond energy of formalin = -130.832kcal/mol, ligand LZ9 301 [A] = -120.118kcal/mol, and fipronil = -101.069 kcal/mol, according to the in silico test results. Formalin has the ability to increase ROS production and affect CDK1 protein more than LZ9 and fipronil ligands, according to the above test results. The findings of the in silico test utilizing the pkCSM online tool software demonstrate that formyl tetrahydrofolate molecules are hazardous and quickly enter and absorb into the human body.

***Key words**: Toxic chemical, CDK1, Formalin, LZ9 ligand, In silico, QSAR.*

Intruduction

Formalin is a colorless, pungent-smelling solution made up of formaldehyde and water. Formalin is rapidly dispersed across the body, including the muscles, intestines, liver, and other tissues (Dorokhov *et al*., 2018; Kamps *et al*., 2019). The enzyme formaldehyde dehydrogenanse can convert formaldehyde to formic acid very quickly, which is then excreted to produce active formyl tetrahydrofolate. Formic acid, on the other hand, is metabolized more slowly and hence accumulates in

the blood (Zhang *et al*., 2019). Metabolic acidosis is caused by low bicarbonate levels and a low pH in the body. The enzyme formyl-tetrahydrofolate-synthetase (formyl-THF-synthetase) in combination with tetrahydrofolate can then eliminate formic acid to form 10-formyl-tetrahydrofolate (10-formyl-THF). Formyl-THF-dehydrogenase catalyzes the conversion of 10-formyl-THF into carbon dioxide and vapor (F-THF-DH)(Reingruber and Pontel, 2019).

When formaldehyde is used as a food preservative and it does not meet health standards, it can damage cellular proteins and nucleic acids (Vartiainen, 2019; Chen *et al*., 2016). This damage can alter cell function, resulting in damage to tissues, organs, and the organism as a whole (Gonzalez-Rivera *et al*., 2020; Hasnidar *et al*., 2020). Formaldehyde is made up of the organic formaldehyde, which is a source of reactive oxygen species (ROS) and can cause oxidative stress(Dhalila *et al*., 2017; Kusumaningrum, 2019). In the oocyte, oxidative stress may cause severe DNA damage. There will also be an increase in apoptosis, as well as a decrease in the capacity of the mitochondrial membrane, BCL-XL, and cytochrome C release (Zhou, 2019). Maturation promoting factor (MPF) and mitogen-activated protein kinase (MAPK) play a role in mammalian organisms. The development of the meiotic oocyte cell cycle is influenced by this protein. CDK1 and cyclin B1 form a heterodimer known as maturation promoting factor (Cao, 2020; Kasman *et al*., 2020).

Cyclin-dependent kinases-1 (CDK1) is a member of the cyclin-dependent protein kinase (CDK) family of enzymes that plays a role in the maturation of unfertilized Xenopus eggs (£ukasik *et al.,* 2021). Cyclin-dependent kinases (CDKs) are a family of protein kinases that help eukaryotic cells go through the cell cycle. Cyclin-dependent protein kinase is involved in cycle-independent processes such as DNA damage repair, epigenetics, stemness, metabolism, and transcription function, in addition to its involvement in cell cycle regulation (Menon, 2018; Michowski, 2020).

The aim of this study is to use a computer program to determine the effect of formalin (formyl tetrahydrofolate) on CDK1 protein during the maturation phase of oocyte cells using an in silico quantitative structure activity relationship (QSAR) test. The QSAR method is used to predict a compound's biological activity as well as endpoint data including toxicity and pharmacokinetic properties (absorption, distribution, metabolism, and excretion = ADME). Carcinogenicity, mutagenicity, hepatotoxicity, teratogenicity, and effects on the reproductive system are all toxicity properties with several complex aspects and no specific mechanism. Most researchers' current research focuses on mutagenicity and carcinogenicity, which are toxicity endpoints (Purwaniati, 2020).

A comparison compound with a similar effect is needed to demonstrate the level of formaldehyde toxicity to CDK1 protein in the maturation phase of oocyte cells. Fipronil is used as a reference compound because it has been shown to induce apoptosis and interrupt the cell cycle in porcine oocytes during maturation in vitro (Chen *et al.,* 2016). Human CDK1/CyclinB1/CKS2 With Inhibitor (PDB ID: 4Y72) are ligands or molecules that have shown good biological activity and can bind to the desired biological target in the docking phase. Formalin is thought to cause a rise in reactive oxygen species (ROS), which leads to oxidative stress. The effect on changes in CDK1 protein, which plays a role in oocyte maturation, necessitates the use of an in silico test to investigate this problem. Modern in silico approaches offer a cost-effective supplement to in vitro models for predicting mammalian metabolism, toxicity, and exposure to pesticide residues or other metabolites before they are synthesized (Clark, 2018).

Docking the molecules whose behavior would be predicted on the selected target cells is how the in silico test is carried out. Docking is the process of aligning small molecules called ligands with a large protein molecule called a target cell. The bond energy value, also known as the Rerank Score, is the product of the in silico test (RS). The amount of energy needed to form bonds between ligands and receptors is referred to as bond energy. The bond would be more stable if the bond energy is lower. It can be expected that the more stable the ligand-receptor bond is, the higher the activity would be (Kesuma *et al*., 2018). As a result, determining the toxicity of formaldehyde (Formyl Tetrahydrofolate) to CDK1 protein in the oocyte maturation phase in silico is important.

Materials and Methods

Materials

The3D structure (PDB ID: 4Y72) Human CDK1/

CyclinB1/CKS2 with inhibitor (LZ9 ligands) downloaded from http://www.rcsb.org/pdb/home.do. Then the 3-dimensional structure of formyl tetrahydrofolate and fipronil was downloaded from https://pubchem.ncbi.nlm.nih.gov/compound/. **Tools** : A computer with Windows 8 64 bit specifications and the Chem Draw Professional 16.0 program, Chem3D 16.0, and Molegro Virtual Docker 5.

Methods

Activity Prediction (Molecular Docking)

The compounds to be docked are formyl tetrahydrofolate, LZ9 ligands, and fipronil, which are drawn 2-D structures using ChemDraw Professional 16.0.then converted to 3-D using Chem3D 16.0 and determined the most stable conformation. After measuring the minimum energy, it is stored in the form of mol2 {SYBYL2 (*. Mol2)}. Structure (PDB ID: 4Y72) Human CDK1/CyclinB1/CKS2 with inhibitor obtained from Protein Data Bank. The results obtained are in the form of a Rerank Score (RS), which is the energy needed in the ligand-receptor interaction process, and from this value, the toxicity activity of formyl tetrahydrofolate can be predicted.

Prediction of Physicochemical, Pharmacokinetic, and Toxicity of Compounds (pkCSM)

The pkCSM online tool was used to predict physicochemical parameters such as Molecular Weight (BM), logarithm of octanol/water partition coefficient (Log P), number of rotating bonds between atoms (Torsion), Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), and Polar Surface Activity (PSA). The pkCSM online tool was also used to perform QSAR approaches to estimate pharmacokinetic properties (ADME: absorption, distribution, metabolism, and excretion) and toxicity of formyl tetrahydrofolate, LZ9 ligand, and fipronil (Liu, 2018). First, a 2-D molecular structure of formyl tetrahydrofolate, LZ9 ligand, and fipronil was constructed in Chem Draw Professional 16.0, then copied to Chem3D 16.0 to build a 3-D structure, and finally saved as a file*.sdf. Second, using the Online SMILES Translator, the structure of formyl tetrahydrofolate, LZ9 ligand, and fipronil was translated into SMILES format (https: //cactus.nci.Nih.gov/translate/). To estimate ADME and compound toxicity, the compounds are processed using the pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/prediction) in this SMILES format. The Protox online tool (http://tox.charite.de/tox/) is used to predict oral toxicity (LD50) in a globally harmonized system (GSH) (Krihariyani *et al*., 2018; Banerjee *et al*., 2018).

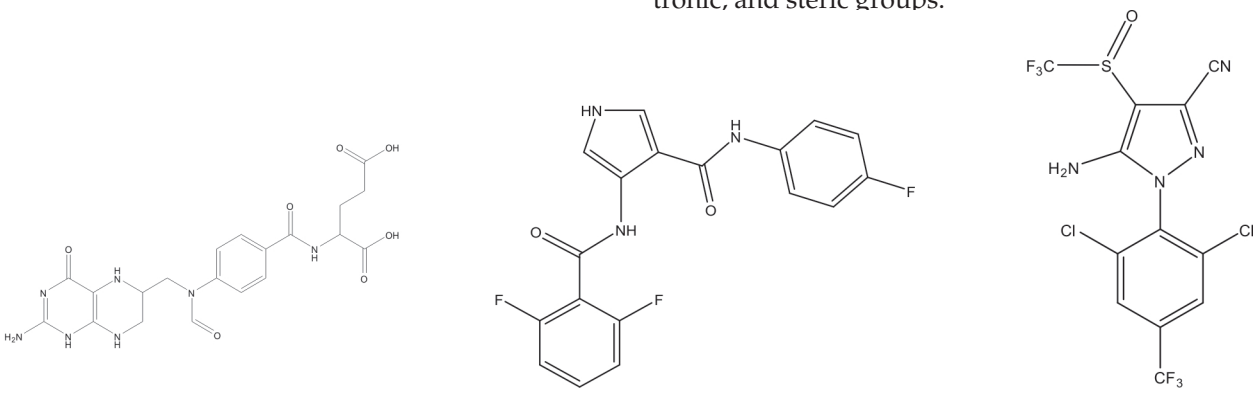
Results

Figure 1 shows the effects by using ChemDraw Professional 16.0 to create a 2-D structure. Chem3D 16.0 is then used to construct a 3-D structure from 2D structures. Figure 2 depicts the next 3D structure that is used at all levels of docking.

Activity Prediction with Docking and Amino Acid Analysis

Figure 3 depicts the interaction of the ligand and receptor on the 4Y72 [A] protein.

Figure 4 and Table 1 show the amino acid residues generated when formyl tetrahydrofolate, LZ9 ligand, and fipronil interact with the protease receptor 4Y72[A], which are bound to hydrogen, electronic, and steric groups.



(a) (b) (c)

Fig. 1. The 2-dimensional structure of the compound (a) formyl tetrahydrofolate;(b) ligand LZ9;(c) fipronil

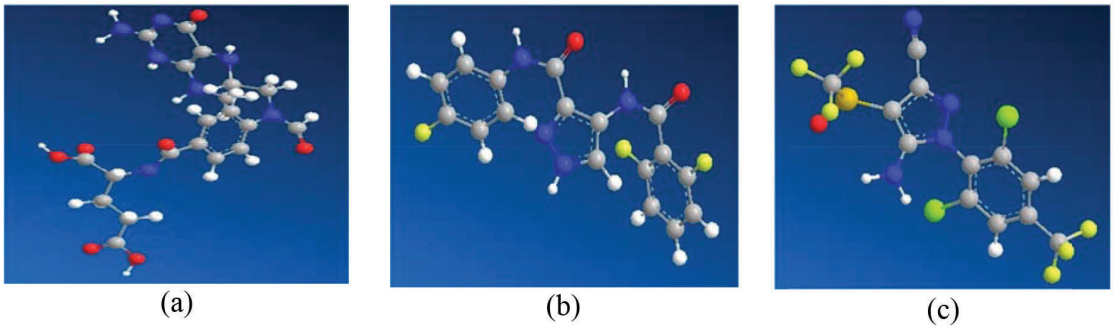


Fig. 2. The 3-D structure stored in SYBYL2 (a) formyl tetrahydrofolate; (b) ligand LZ9;(c) fipronil

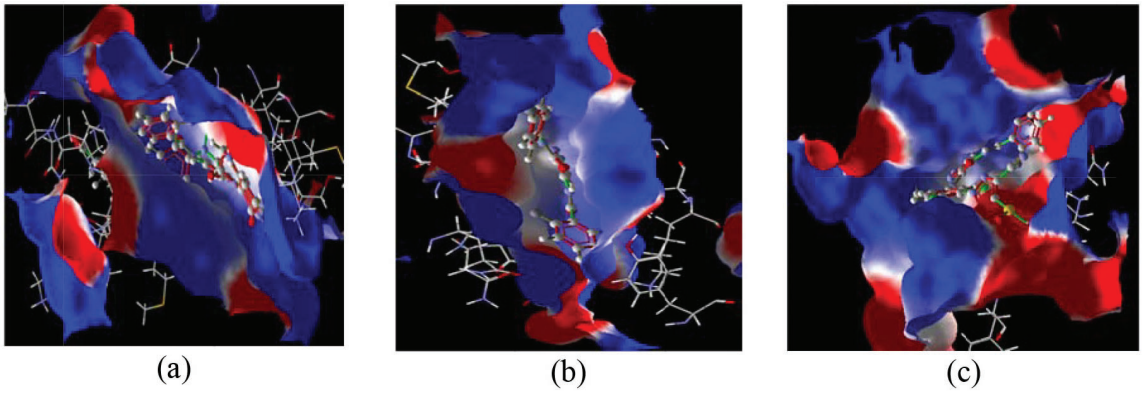


Fig. 3. Receptor interactions with the ligand (a) formyl tetrahydrofolate;(b) ligand LZ9; (c) fipronil

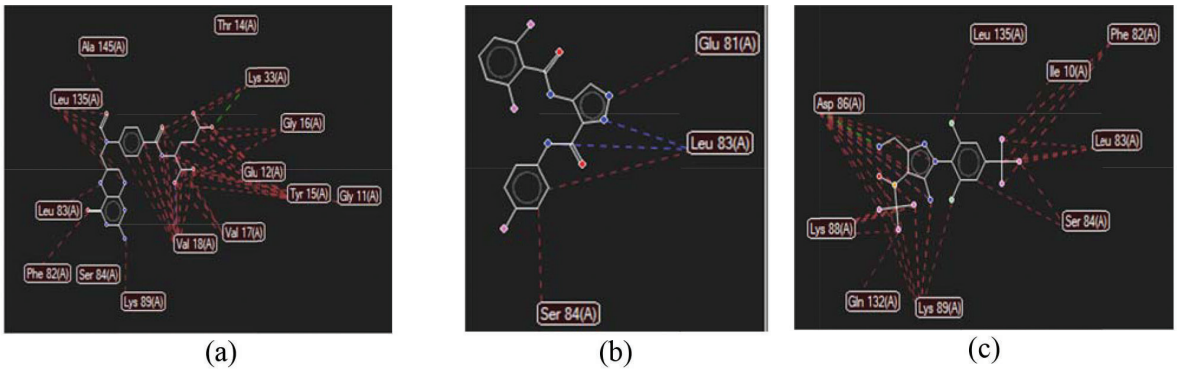


Fig. 4. Amino acid residues on chemical interactions with the protease receptor 4Y72 (a) formyl tetrahydrofolate; (b) LZ9 ligand; and (c) fipronil. (H-Bond, electronic, steric)

Table 2 Shows the outcomes of re-docking formyl tetrahydrofolate, LZ9 ligands, and fipronil with the protease receptor 4Y72 [A].

Compound Physicochemical, Pharmacokinetic, and Toxicity Prediction (pkCSM)

Table 3 shows the effects of in silico parameter prediction for the physicochemical properties of formyl tetrahydrofolate, LZ9 ligands, and fipronil.

Table 4 shows the effects of in silico pharmacokinetic properties (ADME) and toxicity predictions for formyl tetrahydrofolate, LZ9 ligands, and fipronil.

Discussion

Prediction of Activity (Molecular Docking)

The cyclin-dependent kinase 1 (CDK1) protein, cyclin-dependent kinases regulatory subunit 2 (CKS2), and G2/mitotic-specific cyclin-B1 (PDB

Table 1. Amino acid residues on chemical interactions with the protease receptor 4Y72 (a) formyl tetrahydrofolate; (b) LZ9 ligand; and (c) fipronil. (H-Bond, electronic, steric)

Ligand	Amino acid residues and hydrogen bonds		Amino acid residues and electrostatic interactions		Amino acid residues and steric interactions	
Formyl tetrahydrofolate	1	Val 18(A)	1	Lys 33(A)	14	Ala 145(A) Leu 135(A) Leu 83(A) Phe 82(A) Ser 84(A) Lys 89(A) Lys 33(A) Thr 14(A) Gly 16(A) Val 18(A) Gly 11(A) Tyr 15(A) Val 17(A) Glu 12(A)
LZ9	1	Leu 83(A)	0	-	3	Glu 81(A) Leu 83(A) Ser 84(A)
Fipronil	1	Asp 86(A)	1	Asp 86(A)	9	Leu 135(A) Leu 83(A) Ile 10(A) Phe 82(A) Ser 84(A) Lys 88(A) Asp 86(A) Gln 132(A) Lys 89(A)

Table 2. Shows the results of re-docking with Molegro Virtual Docker.

Re docking/Ligand	Formyl tetrahydrofolate	LZ9	Fipronil
I	-126.722	-120.599	-99.5991
II	**-130.832**	**-120.118**	**-101.069**
III	-123.026	-119.591	-99.6858

code: 4Y72) with ligand LZ9 301[A] were used as target molecular receptor structures [{[(2,6-difluorophenyl)carbonyl]amino}-N-(4-fluorophenyl)-1H-pyrazole-3-carboxamide]. The key target receptor for the LZ9 301 [A] ligand in the inhibition of oocyte maturation is cyclin-dependent kinase 1 (CDK1), which can be found in a protein data bank used for molecular modelling(Michowski, 2020; Zhou *et al*., 2019).

This molecular modeling is needed in order to predict the physical chemical properties of ligand molecules as well as to understand the description of compounds that interact with receptors. The interaction between the target molecular receptors and the ligands generates a bond energy value, which can be determined using the docking technique. The amount of energy needed to form bonds between ligands and receptors is referred to as bond energy. The bond would be more stable if the bond energy is lower. It can be expected that the more stable the ligand-receptor bond is, the higher the activity would be (Kesuma *et al*., 2018).

Docking and amino acid analysis are used to predict activity

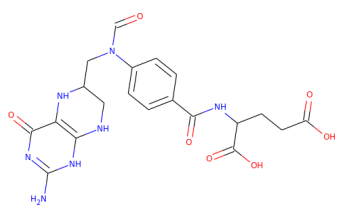
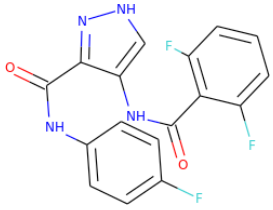
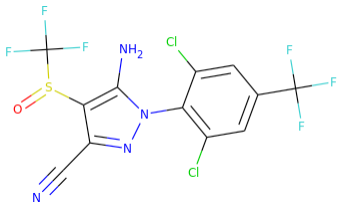
The receptor is imported into the Molegro Virtual Docker software to see its binding energy, which was downloaded from the Protein Data Bank code 4Y72. The discovery of ligand-receptor interacting sites on the 4Y72 [A] protein yielded the following

findings (Figure 3). The cavity with the active ligand LZ9 301[A] is used [Vol = 315,904]. Since it has a region where the original ligand interacts with the protease enzyme, this cavity is used.

Several amino acid residues are produced when the ligand and receptor interact. Figure 4 and Table 1 show the amino acid residues generated when formyl tetrahydrofolate, LZ9 ligand, and fipronil interact with the protein receptor 4Y72[A]. Lipophilic/hydrophobic, electronic, and steric linkages bind amino acid residues. The variation in amino acid residues produced can be noticed in Figure 4 and Table 1 due to changes in the spatial configuration of the structures of the three compounds. Compounds' ability to enter biological membranes is influenced by lipophilic/hydrophobic bonds. The strength of the ligand-receptor interaction and the compound's capacity to penetrate biological membranes are both affected by electronic bonding. The ligand-receptor interaction's compatibility is determined by the steric bond (Bock *et al.*, 2016).

Table 2 shows the findings of re-docking formyl tetrahydrofolate, LZ9 ligand, and fipronil with the protein receptor 4Y72[A]. Formyl tetrahydrofolate has a lower binding energy with the 4Y72[A] receptor than the ligands LZ9 and fipronil. Rerank scores for formyl tetrahydrofolate are -130,832 kcal/mol, ligand LZ9 is -120,118 kcal/mol, and fipronil is -101,06 kcal/mol. According to the Rerank Score, formyl tetrahydrofolate has a lower energy content than the LZ9 ligand and fipronil, indicating that it is more stable in binding to the receptor. This shows

Table 3. Prediction in silico values for the parameters of physicochemical properties of formyl tetrahydrofolate, LZ9 ligands, and fipronil.

Struktur SMILES			
	Formyl tetrahydrofolate	LZ9	*Fipronil*
BM	473.446	360.295	437.152
LogP	-0.7311	3.3316	4.27918
Torsion	10	4	2
HBA	9	3	5
HBD	7	3	1
PSA (A^2)	191.717	144.592	152.897

Information: BM = Molecular Weight; Log P = logarithm of octanol / water partition coefficient; Torsion = bonds between atoms that can rotate; HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors; PSA = Polar Surface Activity.

Table 4. Prediction of in silico pharmacokinetic properties (ADME) and toxicity of formyl tetrahydrofolate, LZ9 ligands, and fipronil Description: VDSS: Steady State of Volume Distribution, BBB: Blood Brain Barrier, CYP2D6: Cytochrome P2D6, Renal OCT2: Renal Organic Cation Transporter 2

ADMET	*Formyl tetrahydrofolate*	LZ9	*Fipronil*
Intestinal absorption (human) (%)	8.91	92.994	85.894
Skin Permeability (log Kp)	-2.735	-2.735	-2.803
VDss *(human)* (log L/kg)	-1.416	-0.508	-0.615
BBB *permeability* (log BB)	-1.706	-1.72	-1.766
CYP2D6 *substrate* (Yes/No)	No	No	No
CYP2D6 *inhibitior* (Yes/No)	No	No	No
Total Clearance (log ml/min/kg)	0.182	-0.486	0.193
Renal OCT2 *substrate* (Yes/No)	No	No	No
AMES *toxicity* (Yes/No)	No	No	Yes
LD50 (mol/kg)	2.568	2.394	2.931

that formyl tetrahydrofolate, unlike LZ9 and fipronil ligands, has the capacity to promote ROS generation and impact CDK1 protein.

Compound physicochemical, Pharmacokinetic, and Toxicity Prediction (pkCSM)

The results of in silico predictions for the Table 3 shows the LZ9 ligands and fipronil. Lipinski *et al.* (1997) studied 2,245 drugs from the World Drugs Index baseline and concluded that compounds with a molecular weight greater than 500, a log value of the octanol / water (log P) partition coefficient of +5, and a donor H-bond are difficult to absorb and have poor permeability (HBD), values of formyl tetrahydrofolate's physicochemical parameters, which has an H-bond acceptor (HBA) with a number of O and N atoms greater than 10 and is represented by the number of O-H and N-H groups greater than 5. Since all values are multiples of five, this analysis is known as Lipinski's rule of five (Abbasi, 2018; Chen *et al*., 2020). Table 3 shows that the compounds of formyl tetrahydrofolate can be identified. Since the molecular weights of the ligand LZ9 and fipronil are less than 500, the logP values are less than 5, and the acceptor and donor values are less than 10, the three compounds are easily absorbed. As formalin levels in the body reach the toxic threshold, it interacts with almost every part of the cell, suppressing cell function, eventually leading to cell death and organ damage.

Table 4 shows the effects of in silico pharmacokinetic properties (ADME) and toxicity predictions for formyl tetrahydrofolate, LZ9 ligands, and fipronil. Chander *et al.* (2017) say that, If the absorption value is greater than 80%, the compound is said to have strong absorption, and if it is less than 30%, it is said to have poor absorption. Orally administered medications are absorbed mostly via the intestine (Chander, 2017). The intestinal absorption (human) value of the formyl tetrahydrofolate compound is less than 30%, while the LZ9 and fipronyl ligands are more than 80% and not less than 30%, respectively, suggesting that the ligand LZ9 and fipronyl compounds have greater absorption than formyl tetrahydrofolate.

A compound is said to have a relatively low skin permeability if its log Kp value is greater than -2.5, according to Chen *et al*. Table 4 shows that the formyl tetrahydrofolate, LZ9 ligand, and fipronil compounds have Skin Permeability (log Kp) values less than -2.5, indicating that the three compounds have strong skin permeability [28]. The volume of distribution (VDSS) is the theoretical volume over which a drug's total dosage must be uniformly distributed in order to achieve the same concentration as blood plasma. The greater the VD content, the more medication is spread in tissues as opposed to plasma.According to Chen *et al.* (2018), a compound has a low Volume Distribution if the Log VD value is -0.15, and a high Volume Distribution if the Log VD value is > 0.45. The VDss (Steady State of Volume Distribution) value of the formyl tetrahydrofolate compound is -1.416, the LZ9 ligand is -0.508, and fipronil is -0.615, as shown in table 4. As a result, it's reasonable to assume that all derivatives of these compounds would be distributed uniformly, resulting in a concentration similar to that found in blood plasma (Chen *et al*., 2018).

The ability of drugs to cross the blood brain barrier is a critical parameter to consider when reducing side effects and toxicity or increasing the effectiveness of drugs with pharmacological activity in the brain.The logarithmic ratio of brain-to-plasma concentrations was used to calculate brain-blood permeability in an animal model in vivo. According to Bagchi *et al.* (2019), a compound will pass the blood brain barrier well if the Log BB value is greater than 0.3, and it cannot be adequately distributed if the Log BB value is less than -1. The log BB value of the formyl tetrahydrofolate compound = -1.706, ligand LZ9 = -1.72, and fipronil = -1.766, as shown in Table 4. Since the log BB value of the three compounds is less than -1, it can be assumed that they can moderate the blood-brain barrier (Bagchi *et al*., 2019).

The majority of metabolic reactions are known to include oxidation mechanisms. Cytochrome P450 is a detoxifying enzyme found mostly in the liver of the human body. It works by oxidizing foreign organic substances, such as medications, and making them easier to excrete. Because inhibitors of these enzymes, such as grapefruit juice, can alter medication metabolism, they are not recommended for cytochrome P450 enzymes. It's crucial to evaluate a compound's potential to inhibit cytochrome P450, which in this case is represented by the isoform of cytochrome P2D6 (CYP2D6). Table 4 shows that formyl tetrahydrofolate, LZ9 ligand, and fipronil had no effect on or inhibition of the CYP2D6 enzyme, implying that these three drugs are processed by P450 enzymes (Wallace and Crosswy, 2017)

The Total Clearance Constant (CLTOT) and the

Renal Organic Cation Transporter 2 (OCT2) substrate can be used to predict the process of compound excretion. CLTOT is a combination of hepatic (metabolism in the liver and bile) and renal clearance (excretion through the kidneys). This has to do with bioavailability, and determining the dose amount is crucial for obtaining steady-state concentrations. Table 4 shows that the CLTOT value of formyl tetrahydrofolate = 0.182, LZ9 ligand = -0.486, and fipronil = 0.193, and that the excretion rate of the three chemicals can be anticipated using these values (Bhattacharya and Patel, 2021). Organic Cation Transporter 2 is a kidney transporter that is involved in the disposition and elimination of medications and other endogenous substances. When used with OCT2 inhibitors, OCT2 substrate has the potential to create adverse effects. Table 4 shows that the three compounds have no effect on the OCT2 substrate, leading to the conclusion that the generated compounds are not OCT2 substrates (Boof *et al*., 2020).

The Ames Toxicity Test can be used to assess a compound's toxicity. The Ames Toxicity Test is a commonly used tool for determining a compound's mutagenic potential using bacteria. A positive test result means the substance is mutagenic and therefore potentially carcinogenic. Fipronil is expected to cause mutagenic effects, as shown in Table 4. Meanwhile, mutagenic effects of formyl tetrahydrofolate and LZ9 ligands are not anticipated.An oral in silico toxicity test on mice (LD50) and a classification of the toxicity of the compounds based on the Globally Harmonized System (GSH) were performed using the Protox online tool to complete the prediction of the toxicity of the three compounds. The Lethal Dose 50 percent (LD50) is the amount of chemical that can kill 50 percent of experimental animal groups. Table 4 shows that the three compounds' projected LD50 values in rodents belong to the toxicity Class V GSH ($2000 < LD50 \leq 5000$), indicating that the molecule has a minimal acute toxicity effect[22].

Conclusion

Final thoughts,the formyl tetrahydrofolate bond has a lower energy than the ligands LZ9 and fipronil.In silico using the molecular docking process, the bond energy values show that formyl tetrahydrofolate has a greater potential to increase ROS output and influence the CDK1 protein than the LZ9 ligand and fipronyl. Formyl tetrahydrofolate compounds' physicochemical, pharmacokinetic, and toxicity are expected to be very well absorbed in the intestine, have strong skin permeability, can be distributed equally to provide the same concentration as blood plasma, can moderately penetrate the blood-brain barrier, and are metabolized by the P450 enzyme. using the pkCSM online method, was predicted to have the greatest cytotoxic activity and did not cause mutagenic effects in a certain amount.

Acknowledgment

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Conflict of Interest

There is no conflict of interest in this study

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