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IN SILICO TOXICOLOGICAL ANALYSIS OF ACTIVE COMPOUNDS PRESENT IN SELECTED PESTICIDES SOLD IN SOUTH-WEST NIGERIA

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Abstract

Introduction: *The study investigated the molecular effects of human exposure to commonly used pesticides in Nigeria.* ***Methods:*** *Utilizing computational methods like clustering analysis, toxicokinetic predictions, molecular docking, and molecular dynamic (MD) simulation, various health impacts were identified.* ***Results and Discussion:*** *The results revealed significant gastrointestinal absorption, P-glycoprotein bypass, blood-brain barrier penetration, and cytochrome P450 inhibition for certain pesticide agents. Notably, oxathiapiprolin showed hepatotoxicity, propanil exhibited drug-induced liver injury (DILI), and 2,4-dichloro-phenoxyacetic acid demonstrated carcinogenicity. Respiratory toxicity was predicted for most pesticides, except propanil and N-(2,6-diethylphenyl) acetamide. Molecular targets were identified, such as bifenthrin targeting programmed cell death 1 ligand 1 and Atrazine targeting potassium voltage-gated channel subfamily H member 3. Binding affinities were computed, with oxathiapiprolin showing -6.526 kcal/mol with short transient receptor potential channel 7. Molecular dynamic simulations indicated significant binding energy changes over time. Atrazine's binding with potassium voltage-gated channel subfamily H member 3 exhibited a total binding energy ΔGbind of -39.410 kcal/mol and -49.135 kcal/mol at 0 ns and 100 ns, respectively. Oxathiapiprolin's binding with short transient receptor potential channel 7 showed ΔGbind of -53.481 kcal/mol and -44.122 kcal/mol at 0 ns and 100 ns.* ***Conclusion:*** *This study suggests potential hepatotoxicity and carcinogenicity of certain pesticides, emphasizing the need for environmental monitoring and stringent regulations to safeguard public health.*

INTRODUCTION

The world's population is expected to approach nearly 10 billion by 2050, resulting in greater pressure for enhanced food production, which is a challenge that governments worldwide aim to address (1-2). Predictions indicate potential crop losses ranging from 32% for grains to 78% for fruit crops in the absence of pesticide use (3). Agricultural fields account for approximately 85% of global pesticide usage, primarily employed to safeguard crops from weed infestations, diseases, and insect pests (4).

Pesticides are chemical substances comprising insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, and others, and are vital for safeguarding plants from pests and diseases, as well as protecting humans from vector-borne illnesses (4-7). Given that food is the primary route of pesticide exposure for consumers, residues in food can pose health risks (8). The environmental risks and health effects of many pesticides remain poorly understood, although a study has identified 90 pesticides among 202 substances toxic to the human brain (9). Pesticide residues pose significant hazards to the ecosystem, persisting in the environment for extended periods and contributing to carcinogenic effects (9). However, their residues in food pose significant health risks to consumers, as

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evidenced by their association with various negative health effects, including carcinogenic, dermatological, endocrine, neurological, gastrointestinal, reproductive, and respiratory impacts (5,7,10-15).

Computational studies play a crucial role in assessing the impact of pesticides on both human health and the environment. By employing advanced modeling techniques, researchers can simulate the behavior of pesticides at molecular, cellular, and ecological levels, providing valuable insights into their potential effects. On the human health front, computational studies can predict the interactions between pesticides and biological systems, such as enzymes and receptors, shedding light on their toxicity mechanisms and potential health risks. Furthermore, computational models can simulate the fate and transport of pesticides in the environment, including their degradation pathways, bioaccumulation potential, and ecological impacts. These models can predict the long-term effects of pesticide exposure on ecosystems, helping to guide sustainable agricultural practices and conservation efforts. This information is invaluable for regulatory agencies and policymakers in setting safety standards and guidelines for pesticide use, allowing for informed decision-making to protect both human health and the environment.

Toxicokinetics analysis delves into the absorption through various route of administration, distribution across body systems to the cell, cellular metabolism, excretion from cell with total clearance, and toxicity evaluation (ADMET) of environmental pollutants within organisms, providing insights into their impact on health. Studies utilizing toxicokinetics contribute to environmental health risk assessments, guiding informed regulatory decisions and interventions for the creation of safer ecosystems. Target prediction in environmental health involves identifying biological molecules, such as proteins, that interact with environmental toxins, assisting in understanding toxicity mechanisms (16).

Molecular docking in toxicology is a computational technique used to predict the interactions between small molecules, often drugs or environmental toxins, and target proteins. By simulating the binding of these molecules to specific receptors or enzymes, insights into their potential toxicity or therapeutic effects can be obtained. Molecular dynamics (MD) simulation in toxicology is a powerful tool used to understand the interactions between toxic substances and biological molecules at the atomic level. By employing computational algorithms, the behavior of molecules over time can be simulated, providing insights into how toxins interact with biological systems. These simulations allow to study various aspects of toxicology, such as the binding affinity of toxins to specific receptors or enzymes, the mechanisms of toxicity, and the potential pathways of detoxification or metabolism within the body.

Utilizing computational methods and experimental validation, such as in silico modeling and omics techniques, enables the prediction of potential adverse effects on organisms. This approach informs risk assessments and guides the development of targeted interventions to minimize environmental hazards (17). In Nigeria, where agricultural activities are integral to rural life, the need for pesticides safety training is evident, given the high household participation in agriculture (70%) and livestock (41%) ownership (18). This study's objective was to examine the molecular effects of human exposure to selected commonly used pesticides in Nigeria (Table 1). This research is crucial as it identifies the underlying biological pathways affected by pollutants and suggests potential diseases that might arise from exposure. The results enhance comprehension of how pesticide exposure affects human health at the molecular level.

Table 1. The Common Names, Trade Name and Uses of Some Common Pesticides in Nigeria.

Common Name	Pesticides Trade Name	Active Agent	Uses
Paraquat	Weedex, Bret-P, Dragon, Gramoxone, Paraforce, Weedcrusher, Weedoff, Ravage, etc.	1,1'-dimethyl-4-4'-Bipyridinium dichloride	Broad-spectrum herbicide for contact weed control across all crops.
Sniper	Sniper	Bifenthrin	Provides a lasting residual effect to combat plant-damaging pests effectively.
Propanil	Propanil, Rhonil, Propaforce, Propan, Propacare, etc.	Propanil	Ideal for post-emergence weed control in rice fields.
Oxidiaxone	Riceforce, Ronstar, Unicrown	Oxathiapiprolin	Effective for pre-emergence weed management in rice cultivation.
Alachlor	Alachlor, Lasso, etc.	N-(2,6-diethylphenyl) acetamide	Suitable for pre-emergence weed control in maize and select legume crops.
Glyphosphate	Forceup, Roundup, Delsate, Glyphosphate, Glycel, Rhonasate, Sarosate, Touchdown forte, Wipeout, etc.	N-(phosphonomethyl) glycine	Systemic herbicide designed for general weed suppression prior to land preparation.
2,4-D Amine	Aminoforce, 2,4-D Amine, Delmin-forte, Select, etc.	2,4-dichloro-phenoxyacetic acid	Effective for both pre- and post-emergence control of broadleaf weeds.
Atrazine	Atrazine, Atraforce, Atrataf, Delzine, Xtrazine	Atrazine	Specifically formulated for managing grass weeds in cereal crops.
Lindane	Gammallin-20	Lindane	Widely utilized in veterinary and human medicine for treating ectoparasites and pediculosis.

METHODS

Preparation of Compounds

Chemical information for the active ingredients of the selected pesticide compounds were retrieved from the PubChem database as previously described (19). The obtained structures were saved in SMILES format.

Clustering Analysis

Clustering analysis by hierarchical approach was conducted on the ChemMine webserver utilizing the ligands' SMILES data, as previously described (20).

In Silico Toxicokinetics Prediction

The toxicokinetics (ADMET) properties of the pesticide compounds (ligands) were predicted on the SwissADME webserver for ADME property (21). Additionally, toxicity prediction was carried out using the ADMETSAR server.

In Silico Target Prediction

Target prediction analysis was carried out on the SEA Search Server, using the SMILES data of each ligand, with homo sapiens selected as the target organism (22).

Molecular Docking

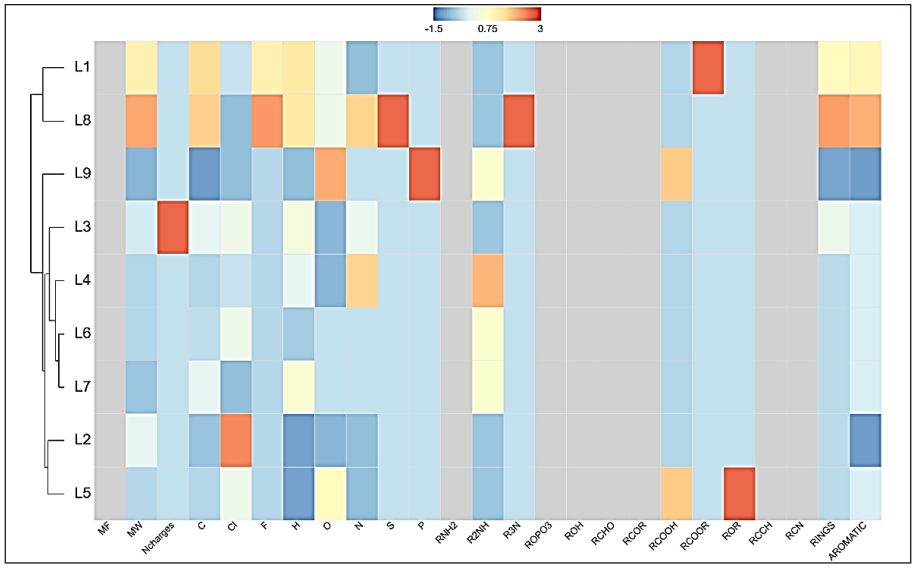
The 3D optimization of ligands' SMILES representations and conversion to .pdb format were performed using ACDLab/Chemsketch and PyMol software, respectively. The 3D structures of the proteins were obtained from the UniProt database in AlphFold pdb format. AutoDock Tools (ADT) v1.5.6 was utilized for formatting ligand and protein structures to pdbqt, followed by molecular docking using AutoDock Vina v1.2.3 (23-25), as detailed previously (26) and the binding affinity was obtained. The binding poses were analyzed using the ezLigPlot application on the ezCADD webserver (27).

Molecular Dynamics Simulation

Desmond from the Schrödinger LLC Package was used to carryout MD simulation 100 nanoseconds simulation time (28-30). Two ligand-protein complexes with top binding affinities from initial docking studies were subjected to simulation steps including preprocessing, and energy minimization, and full-system simulation. The simulations utilized the Optimized Potential for Liquid Simulations (OPLS)-2005 force field under physiological conditions with 0.15 M NaCl counter ions and, employing the NPT ensemble with a temperature of 310 K and pressure of 1 atm and at 100 ps, the trajectories were recorded. Post-simulation analysis of the trajectories gave root-mean-square fluctuation (RMSF), root-mean-square deviation (RMSD), and protein-ligand interaction profile of the complex. Additionally, prime molecular mechanics/generalized born surface area (MMGBSA) was evaluated for calculating binding free energy, considering various energetic contributions (30-32).

RESULTS

The clustering analysis (Figure 1) revealed distinct chemical fingerprint similarities among various compounds, highlighting close resemblances between bifenthrin (L1) and oxathiapiprolin (L8), lindane (L2) and 2,4-dichloro-phenoxyacetic acid (L5), and propanil (L6) and N-(2,6-diethylphenyl)acetamide (L7). Structural representations of the selected toxicants, active ingredients in commonly used pesticides, are illustrated in Figure 2.



Description : L1: Bifenthrin. L2: Lindane. L3: 1,1'-dimethyl-4-4'- Bipyridinium dichloride. L4: Atrazine, L5: 2,4-dichloro-phenoxyacetic acid. L6: Propanil. L7: N-(2,6-diethylphenyl)acetamide. L8: Oxathiapiprolin. L9: N-(phosphonomethyl)glycine.

Figure 1. Cluster of Selected Pesticide Compounds Based On Physicochemical Properties

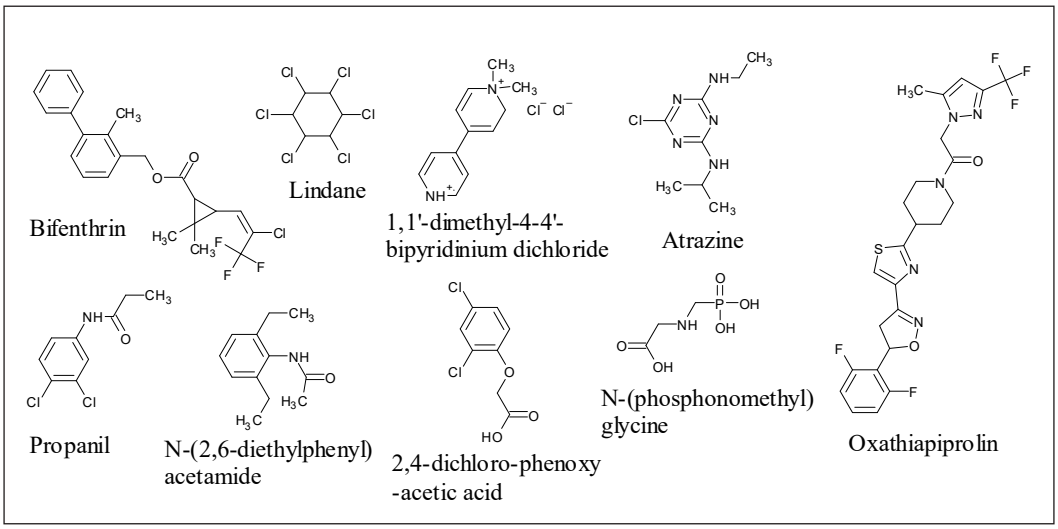


Figure 2. Structural Representation of the Chemical Compounds

Table 2. Predicted Pharmacokinetics Properties of Selected Ligands

SN	Ligands	PubChem CID	Predicted ADME Parameter from SWISSADME											
			MW	MR	TPSA (Å²)	Log P	ESOL Log S	ESOL Class	GIA	P-gp	BBB Permeant	CYPs Inhibitor	Log Kp (cm/s)	BS	SA
1	Bifenthrin	6442842	422.87	108.5	26.3	6.08	-6.09	Poorly soluble	Low	Yes	No	CYP2C19, CYP2C9, CYP2D6	-4.62	0.55	4.12
2	Lindane	727	290.83	57.62	0	3.39	-3.99	Soluble	Low	No	Yes	CYP2C19, CYP2C9	-5.43	0.55	3.68
3	1,1'-dimethyl-4-4'-Bipyridinium dichloride	18179176	259.17	76.24	14.14	-3.19	-3.52	Soluble	Low	Yes	No	NONE	-5.78	0.55	3.31
4	Atrazine		215.68	57.87	62.73	1.65	-2.87	Soluble	High	No	Yes	CYP1A2	-5.76	0.55	2.42
5	2,4-dichloro-phenoxyacetic acid	2256	221.04	49.53	46.53	2.31	-3.12	Soluble	High	No	Yes	CYP1A2	-5.65	0.85	1.91
6	Propanil	1486	218.08	55.58	29.1	2.88	-3.27	Soluble	High	No	Yes	CYP1A2	-5.45	0.55	1.29
7	N-(2,6-diethylphenyl) acetamide	4933	191.27	60.3	29.1	2.52	-2.24	Soluble	High	No	Yes	CYP1A2, CYP2D6	-6.15	0.55	1.4
8	Oxathiapiprolin	85534	539.52	132.32	100.85	4.81	-5.82	Moderately soluble	Low	Yes	No	CYP2C9, CYP2D6, CYP3A4	-6.46	0.55	4.54
9	N-(phosphonomethyl) glycine	56945145	169.07	32.1	116.67	-2.34	1.52	Highly soluble	High	No	No	NONE	-9.75	0.56	2.86

Note: Pharmacokinetics: *Gastrointestinal absorption (GIA), Blood-brain barrier (BBB), P-glycoprotein (P-gp) substrate, Inhibition of Cytochrome P450 (CYPs) type CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, Skin permeation (Log Kp).* ***Lipophilicity****: Consensus Log P.* ***Physicochemical properties****: Molecular weight (MW), Molar Refractivity (MR), Total polar surface area (TPSA).* ***Water Solubility:*** *ESOL Log S, ESOL Class.* ***Medicinal Chemistry:*** *Synthetic accessibility (SA).* ***Druglikeness****: Bioavailability Score (BS)*

The results of toxicokinetic predictions are presented in Table 2. The results showed that some of the pesticide active agents, bifenthrin, lindane, 1,1'-dimethyl-4-4'- bipyridinium dichloride (BDB), and oxathiapiprolin, have high gastrointestinal absorption (GIA); lindane, atrazine, 2,4-dichloro-phenoxyacetic acid, propanil, and N-(2,6-diethylphenyl)acetamide (NDA) could penetrate blood-brain barrier (BBB); bifenthrin, BDB, and NDA could bypass the P-glycoprotein (P-pg) and the majority could inhibit cytochrome P450s (CYPs) such as CYP1A2, CYP2D6 and CYP2C9, except BDB and N-(phosphonomethyl) glycine. The results indicated hepatotoxicity effect of oxathiapiprolin, drug-induced liver Injury (DILI) effect of propanil and oxathiapiprolin, carcinogenicity of 2,4-dichloro-phenoxyacetic acid, and that only propanil and NDA showed no predicted respiratory toxicity (Table 3).

Table 3. Predicted Toxicity of Selected Ligands from Admet 2.0 Server

Toxicity	A	B	C	D	E	F	G	H
hERG Blockers	---	---	---	---	---	---	+	---
Human hepatotoxicity	+	---	-	---	-	-	+++	-
DILI	++	---	---	---	+++	-	+	---
AMES Toxicity	---	--	---	---	---	+	-	---
Rat Oral Acute Toxicity	+	--	+	---	-	---	+++	---
FDAMDD	-	---	+++	---	---	---	+++	++
Skin Sensitization	---	---	+++	---	--	-	---	-
Carcinogenicity	---	--	--	++	--	-	--	--
Eye Corrosion	---	---	---	-	---	---	---	+++
Eye Irritation	--	+++	---	+++	+	--	---	++
Respiratory Toxicity	+++	++	+++	++	---	---	++	++

Note: *A: Bifenthrin B: Lindane. C: Atrazine. D: 2,4-dichloro-phenoxyacetic acid. E: Propanil. F: N-(2,6-diethylphenyl)acetamide. G: Oxathiapiprolin. H: N-(phosphonomethyl)glycine. Symbols denote: 0-0.1(---), 0.1-0.3(--), 0.3-0.5(-), 0.5-0.7(+), 0.7-0.9(++), and 0.9-1.0(+++). DILI: Drug-Induced Liver Injury. FDAMDD: Food and Drug Administration Maximum (Recommended) Daily Dose [+ve, ≤ 0.011 mmol/kg bw/day; -ve, > 0.011mmol/kg bw/day]*

Table 4. Target Prediction Results Showing the P-value and MaxTC

Query	Target Key	Uniprot ID	Target Name	Description	P-value	MaxTC
Bifenthrin	PD1L1_HUMAN	Q9NZQ7	CD274	Programmed cell death 1 ligand 1	2.086e-69	0.36
Lindane	GBRB1_HUMAN	P18505	GABRB1	Gamma-aminobutyric acid receptor subunit beta-1	1.645e-26	1.00
	GBRR1_HUMAN	P24046	GABRR1	Gamma-aminobutyric acid receptor subunit rho-1	1.11e-16	1.00
	GBRA6_HUMAN	Q16445	GABRA6	Gamma-aminobutyric acid receptor subunit alpha-6	1.311e-09	1.00
	GBRA1_HUMAN	P14867	GABRA1	Gamma-aminobutyric acid receptor subunit alpha-1	0.02462	1.00
	GBRB3_HUMAN	P28472	GABRB3	Gamma-aminobutyric acid receptor subunit beta-3	0.03032	1.00
	GBRG2_HUMAN	P18507	GABRG2	Gamma-aminobutyric acid receptor subunit gamma-2	0.05388	1.00
Atrazine	KCNH3_HUMAN	Q9ULD8	KCNH3	Potassium voltage-gated channel subfamily H member 3	2.553e-29	0.39
	PDE4A_HUMAN	P27815	PDE4A	cAMP-specific 3',5'-cyclic phosphodiesterase 4A	2.233e-07	0.48
2,4-dichloro-phenoxyacetic acid	PD2R2_HUMAN	Q9Y5Y4	PTGDR2	Prostaglandin D2 receptor 2	1.584e-104	0.64
	PPARD_HUMAN	Q03181	PPARD	Peroxisome proliferator-activated receptor delta	8.889e-86	0.60
	KMO_HUMAN	O15229	KMO	Kynurenine 3-monooxygenase	1.003e-84	0.37
	PE2R1_HUMAN	P34995	PTGER1	Prostaglandin E2 receptor EP1 subtype	4.75e-73	0.39
Propanil	PLCB_HUMAN	O15120	AGPAT2	1-acyl-sn-glycerol-3-phosphate acyltransferase beta	9.903e-72	0.52
N-(2,6-diethylphenyl) acetamide	MTR1A_HUMAN	P48039	MTNR1A	Melatonin receptor type 1A	2.759e-47	0.38
	MTR1B_HUMAN	P49286	MTNR1B	Melatonin receptor type 1B	1.273e-39	0.38
	HMGB1_HUMAN	P09429	HMGB1	High mobility group protein B1	1.444e-23	0.29
	MYG_HUMAN	P02144	MB	Myoglobin	4.1e-19	0.37
Oxathiapiprolin	TRPC7_HUMAN	Q9HCX4	TRPC7	Short transient receptor potential channel 7	1.312e-29	0.30
	RET4_HUMAN	P02753	RBP4	Retinol-binding protein 4	2.037e-17	0.30
N-(phosphonomethyl) glycine	GGH_HUMAN	Q92820	GGH	Gamma-glutamyl hydrolase	3.081e-26	0.32

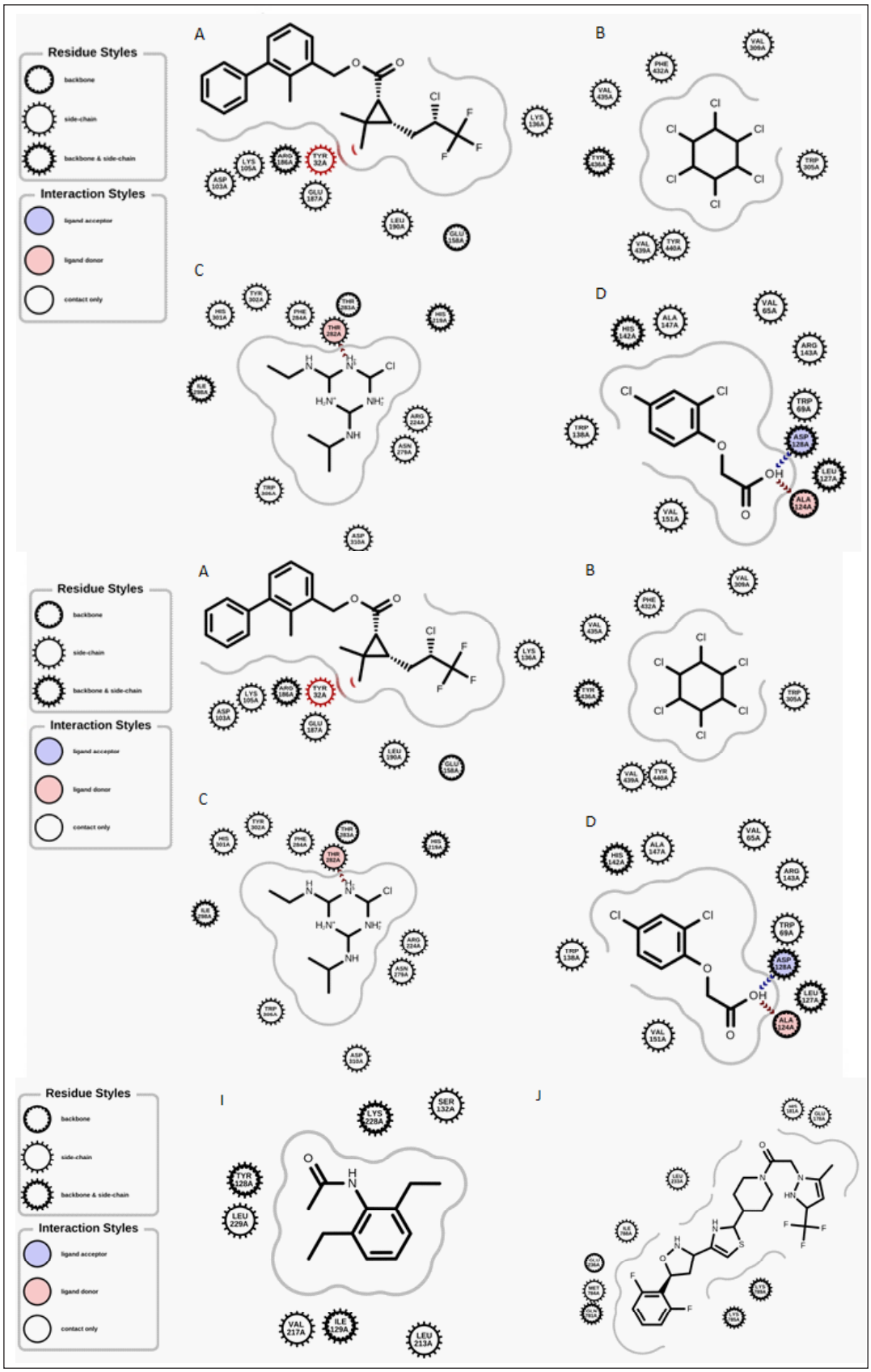
Table 5. Molecular Docking Parameters with Binding Affinity

SN	Target Protein	Binding affinity (kcal.mol^{-1})							
		A	B	C	D	E	F	G	H
1	Programmed cell death 1 ligand 1 (Q9NZQ7).	-4.527							
2	Gamma-aminobutyric acid receptor subunit beta-1 (P18505)		-4.085						
3	Gamma-aminobutyric acid receptor subunit rho-1 (P24046)		-4.176						
4	Gamma-aminobutyric acid receptor subunit alpha-6 (Q16445)		-4.503						
5	Potassium voltage-gated channel subfamily H member 3 (Q9ULD8)			**-5.661**					
6	cAMP-specific 3',5'-cyclic phosphodiesterase 4A (P27815)			-4.049					
7	Prostaglandin D2 receptor 2 (Q9Y5Y4)				-4.615				
8	Peroxisome proliferator-activated receptor delta (Q03181)				-4.581				
9	Kynurenine 3-monooxygenase (O15229)				**-5.11**				
10	1-acyl-sn-glycerol-3-phosphate acyltransferase beta (O15120)					-4.695			
11	Melatonin receptor type 1A (P09429)						-4.815		
12	High mobility group protein B1(P48039)						**-5.345**		
13	Short transient receptor potential channel 7 (Q9HCX4)							**-6.526**	
14	Gamma-glutamyl hydrolase (Q92820)								-3.213

Note: *A: Bifenthrin B: Lindane. C: Atrazine. D: 2,4-dichloro-phenoxyacetic acid. E: Propanil. F: N-(2,6-diethylphenyl)acetamide. G: Oxathiapiprolin. H: N-(phosphonomethyl)glycine*

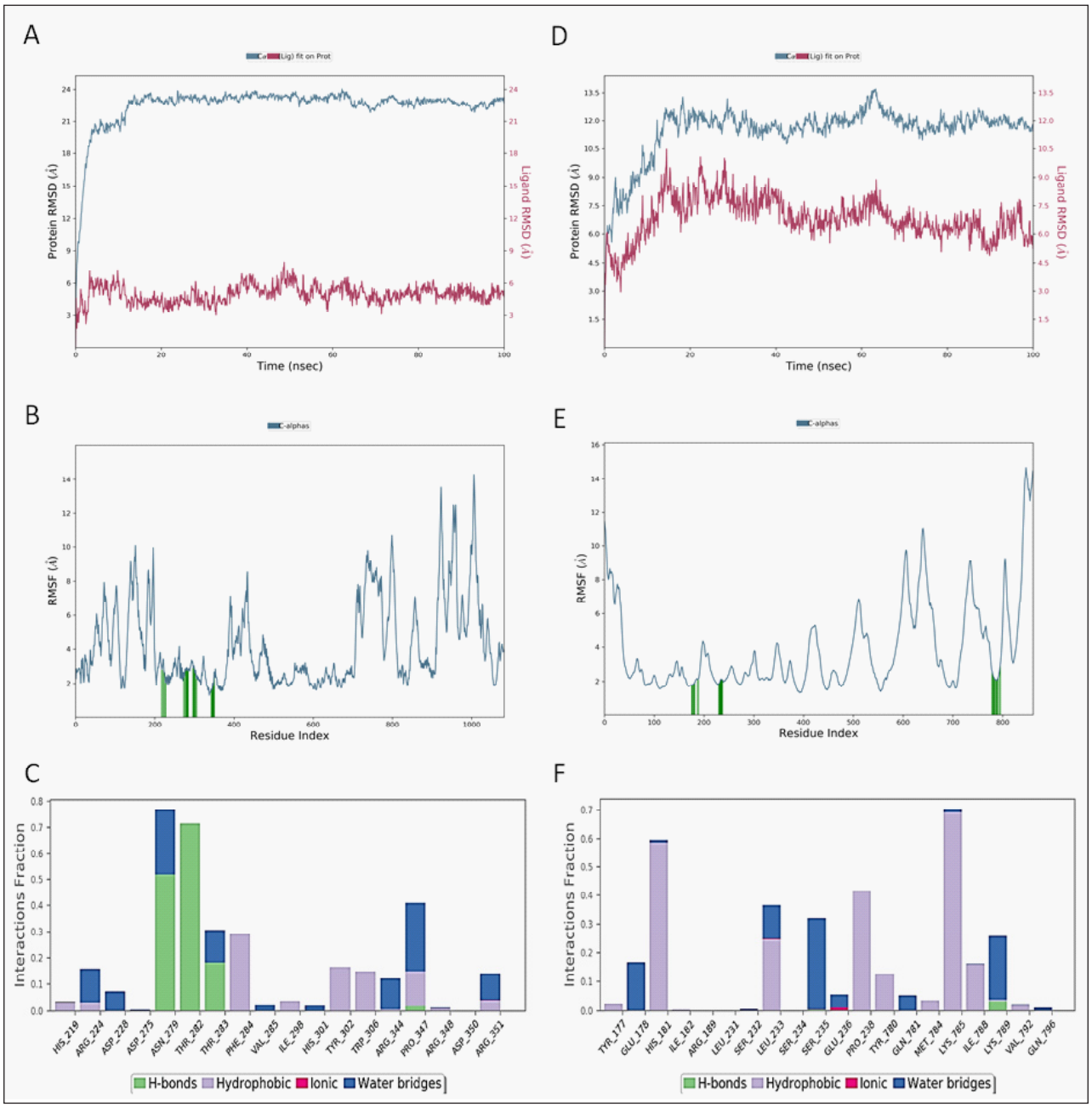
Target prediction results (Table 4) indicated specific molecular targets for each compound. Bifenthrin targeted programmed cell death 1 ligand 1; lindane targeted several gamma-aminobutyric acid receptor subunits, atrazine targeted potassium voltage-gated channel subfamily and others; 2,4-dichloro-phenoxyacetic acid targeted prostaglandin D2 receptor 2 and others; propanil targeted 1-acyl-sn-glycerol-3-phosphate acyltransferase beta, N-(2,6-diethylphenyl) acetamide targeted several melatonin receptor types, oxathiapiprolin targeted short transient receptor potential channel 7 and others, while N-(phosphonomethyl) glycine targeted gamma-glutamyl hydrolase.

The molecular docking results (Table 5) showcase the binding affinities of interactions between ligands and protein targets. The binding affinity of oxathiapiprolin interaction with short transient receptor potential channel 7 was -6.526 kcal/mol, atrazine with potassium voltage-gated channel subfamily H member 3 (-5.661 kcal/mol), and N-(2,6-diethylphenyl) acetamide with high mobility group protein B1 (-5.345 kcal/mol). The structural presentation of these docking interactions is depicted in Figure 3.



Description: (A) Bifenthrin and programmed cell death 1 ligand 1. (B) Lindane and gamma-aminobutyric acid receptor subunit alpha-6. (C) Atrazine and potassium voltage-gated channel subfamily H member 3. (D) 2,4-dichloro-phenoxyacetic acid and prostaglandin D2 receptor 2. (E) 2,4-dichloro-phenoxyacetic acid and peroxisome proliferator-activated receptor delta. (F) 2,4-dichloro-phenoxyacetic acid and kynurenine 3-monooxygenase. (G) Propanil and 1-acyl-sn-glycerol-3-phosphate acyltransferase beta. (H) N-(2,6-diethylphenyl)acetamide and melatonin receptor type 1A. (I) N-(2,6-diethylphenyl)acetamide and high mobility group protein B1 (J) Oxathiapiprolin and short transient receptor potential channel 7

Figure 3. Docking Interaction



Description: (A) RMSD of atrazine and potassium voltage-gated channel subfamily H member 3 (Q9ULD8) (B) RMSF of Q9ULD8 on binding to atrazine. (C) Interaction profile of atrazine with Q9ULD8. (D) RMSD of oxathiapiprolin and short transient receptor potential channel 7 (Q9HCX4). (E) RMSF of Q9HCX4 on binding to oxathiapiprolin (F) Interaction profile of oxathiapiprolin with Q9HCX4

Figure 4. MD Simulation Results

The results of MD simulations, illustrated in Figure 4, offer valuable insights into the dynamic behavior and interactions of protein-ligand complexes under realistic conditions. Atrazine binding to potassium voltage-gated channel subfamily H member 3 (Q9ULD8) demonstrated stability, with an RMSD of 24.0 Å for the protein and 8 Å for the ligand across 0-100 ns (Figure 4a). The RMSF of Q9ULD8 indicated minimal fluctuation at specific amino acid residues (Figure 4b), while protein-ligand interactions involved various residues, including ASN279, THR282, THR283, PHE284, ARG344, and PRO347, encompassing hydrophobic interactions, hydrogen bonds, water bridges, and ionic interactions (Figure 4c).

Similarly, oxathiapiprolin binding to short transient receptor potential channel 7 (Q9HCX4) displayed stability, with an RMSD of 13 Å for the protein and 10.5 Å for the ligand over 0-100 ns (Figure 4d). The RMSF of Q9HCX4 exhibited minimal fluctuation at specific amino acid residues and the N-terminal region (Figure 4e), with protein-ligand interactions involving residues such as HIS181, LEU233, SER235, PRO238, LYS785, and LYS789, encompassing various interactions (Figure 4f). A detailed schematic of ligand atom interactions with protein residues is presented in Figure 5, validating the amino acid residues involved in docking interactions.

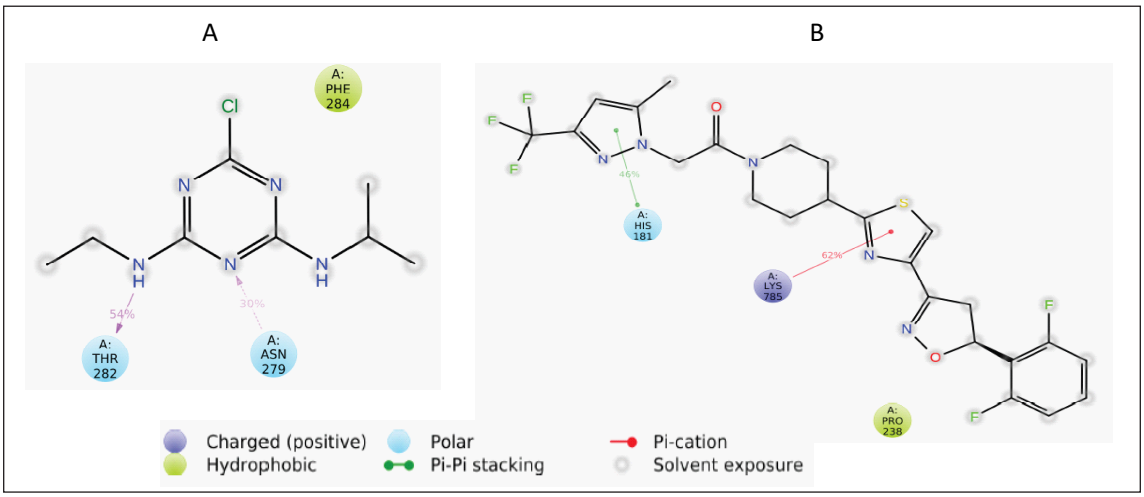


Figure 5. A representation of the Interactions of (A) Atrazine and Q9ULD8 (B) Oxathiapiprolin and Q9HCX4

Table 6. Prime MMGBSA Binding Energy of Protein-Ligand Interaction Before and After Molecular Dynamics Simulation

Complex	Simulation Time (ns)	MMGBSA ΔG (kcal.mol^{-1})							
		Coulomb	Covalent	Hbond	Lipo	Packing	Solv_GB	vdW	ΔG^{bind} (Total)
Atrazine and Potassium voltage-gated channel subfamily H member 3 (Q9ULD8)	0	-6.945	1.504	-0.980	-16.600	-0.843	12.533	-28.078	-39.410
	100	-11.571	0.759	-1.570	-21.400	-0.134	14.389	-29.608	-49.135
Oxathiapiprolin and Short transient receptor potential channel 7 (Q9HCX4)	0	0.498	1.197	-0.016	-14.533	-1.282	7.228	-46.575	-53.481
	100	8.654	1.478	0	-13.155	-1.010	5.390	-45.479	-44.122

***Note: Covalent**: **Solv GB**: Generalized Born electrostatic solvation energy. Covalent binding energy. **Coulomb**: Coulomb energy. **Hbond**: Hydrogen bonding energy. **Packing**: Pi-pi packing correction. **vdW**: Van der Waals energy. **Lipo**: Lipophilic energy. **Total**: Total energy (Prime energy)*

Furthermore, the computed binding free energies using MMGBSA (Table 6) provide insights into the stability and energetics of protein-ligand interactions throughout the simulation. Notably, atrazine's binding with Q9ULD8 exhibited a total binding energy ΔGbind of -39.410 kcal/mol and -49.135 kcal/mol at 0 ns and 100 ns, respectively, indicating high stability. Conversely, oxathiapiprolin's binding with Q9HCX4 showed a ΔGbind of -53.481 kcal/mol and -44.122 kcal/mol at 0 ns and 100 ns, respectively, suggesting less stable interactions under simulated physiological conditions.

DISCUSSION

Toxicokinetics, also known as ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity), is a fundamental concept in pharmacology and toxicology. It encompasses the study of how toxic substances move through the body and are processed. Absorption refers to the uptake of a substance into the body, often through ingestion, inhalation, or dermal contact. Distribution involves the movement of the substance throughout the body via the bloodstream, where it can accumulate in various tissues or organs. Metabolism, often occurring in the liver, involves the chemical transformation of the substance into metabolites, which may be more or less toxic than the original compound. Excretion refers to the removal of the substance and its metabolites from the body, usually through urine, feces, or sweat. Understanding toxicokinetics is crucial for assessing the potential risks associated with exposure to toxic substances. Factors such as the chemical properties of the substance, route of exposure, and individual variability in metabolism can influence its toxicokinetic profile.

Toxicants with high gastrointestinal absorption (GIA) pose a risk of easy penetration from the intestine into the bloodstream, potentially causing toxicity or damage to internal organs like the liver and kidneys. Atrazine, 2,4-dichloro-phenoxyacetic acid, propanil, N-(2,6-diethylphenyl)acetamide, and N-(phosphonomethyl) glycine, identified with high GIA, also exhibit high blood-brain barrier (BBB) permeability, suggesting the potential toxicity effects on the brain and central nervous system.

P-glycoprotein (P-gp) and Cytochrome P450 (CYP) enzymes play crucial roles in drug metabolism and transport. Ligands showing "Yes" suggest inhibition, while "No" implies no inhibitory effect, pointing to the possibility of a first pass effect, leading to reduced toxicity. P-glycoprotein (P-gp) is a membrane transporter that plays a role in drug absorption and distribution by actively pumping drugs out of cells. P-gp can influence drug bioavailability, and its inhibition or modulation is a consideration in drug development to enhance drug efficacy (30). Changes in the activities of Cytochrome

P450 enzymes (CYPs) can significantly alter the metabolism of drugs, leading to either increased bioavailability or decreased efficacy (33). CYP3A4 is the most abundant CYP enzyme in the liver, metabolizing a broad range of drugs. CYP3A4 interactions are a major consideration in drug development, impacting drug clearance and potential for drug-drug interactions as CYP1A2 metabolizes drugs and xenobiotics. Its role is significant in the clearance of certain drugs, and its induction can affect drug metabolism rates. Moreover, polymorphisms in CYP2D6 and CYP2C19 can lead to variations in drug metabolism, influencing individual drug responses.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine, ATR) is among the most widely used herbicides globally, with extensive application in crops like sugarcane, corn, and sorghum (33-35). Residues of atrazine have been detected in various biological media, including the brain, blood, breast milk, hair, placenta, and urine, indicating its ability to permeate through the placental and blood-brain barriers (35-36). Studies on atrazine neurotoxicity have revealed altered gene expression levels associated with adverse effects on the dopaminergic system, glutamatergic neurons, and astrocytes (35). Moreover, atrazine exposure has been linked to liver and testis gene expression changes, kidney and liver damage, neurodegenerative diseases, and exhibits high oral acute and respiratory toxicity (37-38).

Exposure of human breast epithelial MCF-10A cells to environmentally relevant concentrations of atrazine have shown differential expression of proteins involved in various cellular compartments and functions, including stress response regulation, structural proteins, and oncogenesis proteins (39). Atrazine's weak binding affinity to human serum albumin in aqueous solution compared to 2,4-dichlorophenoxyacetic acid (2,4-D) at physiological pH has implications for the clearance, volume of distribution, and efficacy of 2,4-D, as the free fraction of the compound determines its ability to cross the blood-brain barrier and elicit pharmacological or toxicological effects (40). Exposure to 2,4-D has been associated with dose-dependent inhibition of DNA synthesis, leading to cytoskeletal perturbation, particularly in microtubules and micro-filaments, and alteration of cytoskeletal protein synthesis and composition (40).

N-(phosphonomethyl)glycine, a broad-spectrum herbicide, inhibits EPSP synthase, crucial for amino acid production in plants, resulting in weed death. It has been associated with predicted kidney and liver damage and high respiratory toxicity (41). Oxathiapiprolin, a piperidinyl thiazole isooxazoline fungicide, exhibits excellent control against oomycete fungal diseases, including late blight, root rot, stem rot, downy mildew, and blight. Risk assessment studies indicate that oxathiapiprolin alters the structure of soil fungal communities, particularly affecting Ascomycota and Mortierellomycota (42).

Target prediction in toxicological studies is a critical aspect of assessing the potential risks associated with chemical compounds. It involves identifying the molecular targets within biological systems that may be affected by exposure to these compounds. By understanding the specific targets, researchers can predict the potential adverse effects and design strategies for toxicity testing and risk assessment. Programmed death ligand 1 (PDL1) regulates T cell proliferation and IL-10 secretion, expressed in heart, placenta, lung, and skeletal muscle tissues (43). Programmed cell death protein 1 (PD-1) plays a role as co-inhibitory receptor on antigen-stimulated T cells (44). Gamma-aminobutyric acid receptor subunit beta-1 (GABA) receptors, prevalent in the central nervous system (CNS), is involved in the neuronal firing, emotion regulation, cognition, pain, sleep, and motor function. GABAAR is a significant drug target for neuropsychiatric disorders (44). Genetic studies link GABAAR subunit genes to epilepsy and bipolar disorders (45-46). GABAA receptors at cerebellar Golgi cell/granule cell synapses enhance cognitive processing and responses to the environment (47).

Potassium voltage-gated channel subfamily H member 3, or voltage-gated potassium ion channels (Kv), play a vital role in cellular processes, including excitable cell functioning, apoptosis regulation, neurotransmitter and hormone release, and cardiac activity maintenance. These channels exhibit broad distributions in the nervous system and other tissues (48). CAMP-specific 3',5'-cyclic phosphodiesterase 4A enzymes contribute to cAMP and cGMP homeostasis, with PDE4A potentially regulating anxiety and emotional memory (49).

Prostaglandin D2 receptor 2 (PGD2) binds to receptors PTGDR (DP1) and CRTH2 (DP2), mediating non-inflammatory effects in the brain and mast cells, such as vasodilation, inhibition of cell migration, smooth muscle relaxation, and eosinophil apoptosis (50). Peroxisome proliferator-activated receptor delta (PPAR-δ) is very essential in the pancreatic islet beta cells, promoting insulin secretion and protecting against insulin resistance by regulating energy substrate utilization (51-52).

Kynurenine 3-monooxygenase (KMO) regulates the kynurenine pathway, impacting the synthesis of biologically active metabolites in the brain and immune-mediating cells, including T cells, macrophages, microglia, and hepatocytes (53). 1-acyl-sn-glycerol-3-phosphate acyltransferase beta (PlsC in bacteria) catalyzes

lysophosphatidic acid acylation, producing phosphatidic acid, a precursor for membrane glycerophospholipid synthesis. Some AGPAT isoforms are endoplasmic reticulum transmembrane proteins, contributing to phospholipid production for lamellar body formation (54-55).

Melatonin receptor type 1A, an integral membrane protein, is localized in specific brain regions, such as the hypothalamic suprachiasmatic nucleus for circadian rhythm and the hypophysial pars tuberalis for reproductive effects. Melatonin receptors, G protein-coupled, are expressed in various CNS parts and peripheral organs (56-57). High mobility group protein B1 (HMGB1), a nonhistone nuclear protein, functions as a DNA chaperone in the nucleus and promotes autophagy in the cytoplasm. HMGN proteins, present in vertebrates, control chromatin structure, gene transcription, DNA repair, and cellular development inside the nucleus (58). Short transient receptor potential channel 7 is highly selective for calcium and widely expressed in various tissues, playing roles in calcium regulation (59-60). Short transient receptor potential channel 7's high bonding affinity with oxathiapiprolin suggests potential implications in seizure-induced neuron death due to its role in ion channel activity and cation transport (61).

In this study, binding interactions with an affinity of less than -5.0 kcal/mol were considered indicative of strong binding affinity. Molecular docking was employed to predict ligand binding sites (active and allosteric/regulatory sites) on the surfaces of biological macromolecules (proteins, RNA, or DNA) and to calculate the binding affinities of their interactions (62). A binding energy score of ≤−5.00 kcal/mol indicates good affinity between the target protein and the ligand (63). In toxicology, molecular docking plays a crucial role in understanding how chemicals interact with biological systems at the molecular level. By analyzing the binding affinity and the structure of the resulting complexes, it is possible to predict the likelihood of a compound causing toxic or therapeutic effects. Molecular docking can help elucidate the mechanisms underlying toxicity, thus serving as a valuable tool in toxicological research, facilitating the identification and characterization of toxic compounds and aiding in the design of safer alternatives.

In this study, MD simulation was carried out to assess atomic-level variations in the protein-ligand system and to evaluate the stability of the protein-ligand complex in a dynamic environment (26). MD simulation explores a wide range of conditions and parameters that may not be feasible or ethical to study experimentally. It helps to manipulate variables such as temperature, pressure, pH, and concentration to mimic different physiological environments and investigate how these factors influence toxin behavior. RMSD values exceeding 4 Å indicate relatively large conformational changes in the complexes, suggesting conditions that may be observed in the actual physiological state and potentially impacting gene regulation networks. Understanding these molecular interactions is crucial for predicting the toxicity of chemicals, designing safer drugs, and developing effective treatments for poisoning or environmental contamination.

Prime MM-GBSA consists of various energy properties, reporting energies for the receptor, ligand, and complex structures, as well as energy differences related to strain and binding (26). The more negative the score, the higher the free energy released in complex formation. The total binding free energy confirmed the stability of the complexes under physiological conditions, indicating their reasonable stability. High total energy indicates a strong binding affinity between the molecules. However, the interpretation depends on the specific contributions of individual energy terms and the context of the study.

Atrazine - potassium voltage-gated channel subfamily H member 3 (Q9ULD8) complex showed increased binding affinity at 100 ns than at 0 ns, which is also an indication of effect of some contributory energy such as high coulomb energy sustained and improved interaction in simulated physiological condition. Oxathiapiprolin - short transient receptor potential channel 7 (Q9HCX4) complex showed decreased binding affinity at 100 ns than at 0 ns, which is an indication of effect of some contributory energy such as low coulomb energy that altered simulated physiological condition.

High coulomb energy indicates strong electrostatic interactions between charged groups, which could suggest either strong attraction or repulsion between the binding partners. This could be significant in understanding the stability or specificity of the binding interaction. High covalent binding energy suggests the formation of strong covalent bonds between the binding partners, which could indicate a stable binding complex. However, in drug design, covalent binding can lead to unwanted side effects, so careful consideration is needed. High hydrogen-bonding correction suggests the formation of strong hydrogen bonds between the binding partners, which can contribute to the specificity and stability of the binding interaction.

Moreover, excessively high lipophilic energy might indicate a propensity for non-specific binding to hydrophobic regions. High pi-pi packing correction indicates strong interactions between aromatic rings, which are important in stabilizing binding complexes,

especially in protein-ligand interactions. Moreover, high solvation energy could indicate strong interactions between the solute and solvent molecules, which may affect the overall stability of the binding complex. However, excessively high solvation energy might suggest poor solubility or unfavorable interactions with the solvent. High van der Waals energy indicates strong attractive interactions between non-polar groups, which could contribute to the stability of the binding complex. However, excessively high van der Waals energy might lead to non-specific binding or aggregation.

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CONCLUSION

This study exposes the impact of certain pesticide compounds on biological systems, highlighting potential health risks. The results indicated possible hepatotoxicity effect of propanil and oxathiapiprolin, and carcinogenic effect of 2,4-dichloro-phenoxyacetic acid, and that respiratory toxicity is associated with the use of most of the routine pesticides. The findings emphasize the significant threat these toxicants pose to public health, prompting the need for sustainable environmental monitoring and stringent regulations on some pesticide usage in order to safeguard and protect public health. This research also paves way for future research on strategy for mitigating the effects of these identified toxicants.

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