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ORIGINAL ARTICLE

Computational Tool for Immunotoxic Assessment of Pyrethroids toward Adaptive Immune Cell Receptors

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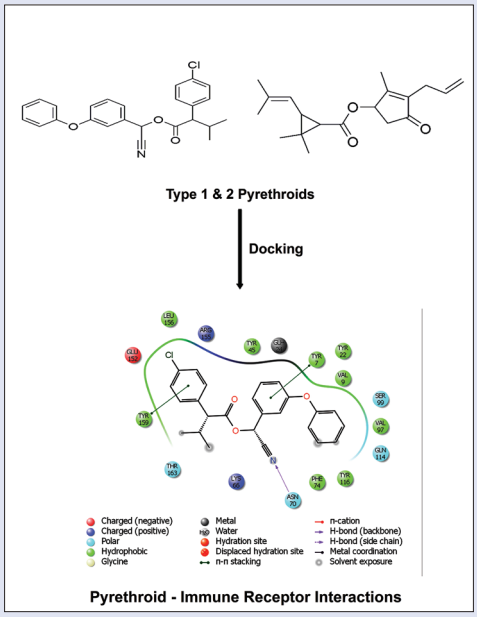
ABSTRACT

Background: Pyrethroids have prominently known for their insecticidal actions worldwide, but recent reports as anticancer and antiviral applications gained a lot of interest to further understand their safety and immunotoxicity. **Objective:** This encouraged us to carry out our present study to evaluate the interactions of pyrethroids toward adaptive immune cell receptors. **Materials and Methods:** Type 1 and Type 2 pyrethroids were tested on T (CD4 and CD8) and B (CD28 and CD45) immune cell receptors using Maestro 9.3 (Schrödinger, LLC, Cambridge, USA). In addition, top-ranked tested ligands were too explored for toxicity prediction in rodents using ProTOX tool. **Results:** Pyrethroids (specifically type 2) such as fenvalerate (–5.534 kcal/mol: CD8), fluvalinate (–4.644 and – 4.431 kcal/mol: CD4 and CD45), and cypermethrin (–3.535 kcal/mol: CD28) have outcome in less energy or more affinity for B-cell and T-cell immune receptors which may later result in the immunosuppressive and hypersensitivity reactions. **Conclusion:** The current findings have uncovered that there is a further need to assess the Type 2 pyrethroids with wet laboratory experiments to understand the chemical nature of pyrethroid-induced immunotoxicity.

Key words: Docking, immunotoxicity, pyrethroids, receptor

SUMMARY

- Fenvalerate showed apex glide score toward CD8 immune receptor, while fluvalinate confirmed top-ranked binding with CD4 and CD45 immune proteins
- In addition, cypermethrin outcame in top glide score against CD28 immune receptor
- Top dock hits (Type 2) pyrethroids have shown probable toxicity targets toward AOFA: Amine oxidase (flavin-containing) A and PGH1: Prostaglandin G/H synthase 1, respectively.



Pyrethroid - Immune Receptor Interactions

Abbreviations used: PDB: Protein Data Bank; AOFA: Amine oxidase (flavin-containing) A; PGH 1: Prostaglandin G/H synthase 1.

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INTRODUCTION

During the past decades, pesticides have recognized as a major environmental chemical pollution in agriculture which eventually resulting in serious health concern.[1-3] Pyrethroid class of pesticides (insecticides) is often in use due to their low tendency to accumulate in organisms and short biodegradation period.[4] Type 1 and Type 2 pyrethroids are basically synthetic analogs derived from main nucleus pyrethrins, an imperative phytochemical entity isolated from the flowering part of *Chrysanthemum cinerariaefolium*. The division of pyrethroids into two classes is based on chemical structural differences and toxicological and neurophysiological actions. The chemical difference showed that Type 2 pyrethroids contain an α-cyano-phenoxy-benzyl moiety while Type 1 pyrethroids devoid of an α-cyano component which in turns relates to the types of poisoning syndrome. Mostly, synthetic pyrethroids exist in different forms of enantiomers due to their chiral nature.[5-7] The Type 1 compounds symptoms include increased reactivity to whole-body tremor, whereas salivation, choreoathetosis, and chronic seizures are quite common with Type 2 pyrethroids.[8,9] The pyrethroids are toxic to insects due to their depolarization action on nerve membranes.[10-12] Photostability, high efficacy at low concentrations, easy disintegration, and low toxicity to birds and mammals are the key rewards of pyrethroid insecticides.[13,14] The products containing pyrethroid insecticides are

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used for the control of pests such as mites, ants, weevils, and beetles on various crops, including cotton, corn, cereals, soybeans, and vegetables and also for the control of endo- and ecto-parasites on animals.[15] Pyrethroid insecticides are a product of choice in many countries due to their expeditious metabolism rate and low toxicity to humans and other nontarget animals. Due to their high potency on a huge number of pests, these have become a premier choice for the control of malaria and other vector-borne diseases.[16-18] For the last two decades, diminution on the sales of organophosphorus insecticides researchers has developed pyrethroids with the merits of more promising insecticidal and antiparasitic formulation.[19,20] Globally, use of pyrethroids has resulted in contamination problem, and moreover, even their metabolites possess a significant role in polluting food and water which ultimately leads to health problems.[17,21] Humans have exposed to pyrethroid insecticides by their well-built use in personal protection such as mosquito nets drenched with pyrethroid insecticides, disinfection of aircraft, in agriculture and public health, respectively.[22-24] Numerous manifestations have observed in the nervous, respiratory, cardiovascular, and gastrointestinal systems which ultimately resulting in allergic reactions, myocardial impairment, and even death due to respiratory failure by the minute subjection of pyrethroids to the human beings.[25] Immunotoxicity of pyrethroid insecticides is still unclear specifically on the adaptive immune system. T- and B-cells play the vital role to provide adaptive immunity against pathogens by producing various cytokines and antibodies. CD4 and CD8 are the surface markers which are present on T-cell receptors whereas CD28 and CD45 are present on B-cell receptors. As the immunotoxicity of pyrethroid insecticides on an adaptive immune system is still unexplored, it is essential to first observe their binding affinity towards the adaptive immune cell receptors. Chiral transformation of pyrethroid compounds may have a different potential toward adaptive immune system receptors. Molecular docking has provided an important tool for estimating the interaction of compounds toward specific receptors along with its pharmacokinetic properties. Outstandingly, ProTOX online tool has too shown promising toxicity prediction output in the form of LD_{50} value against rodents.[26,27] Thus, the main aim of this study is to predict the binding affinity of pyrethroid insecticides toward T-cell (CD4, CD8) and B-cell receptors (CD28, CD45).

MATERIALS AND METHODS

Docking simulations were run on core TM processor with 4 GB RAM and 220 GB with center Linux Enterprise version as the operating system using Maestro 9.3 (Schrödinger, LLC, Cambridge, USA) while toxicity prediction was carried out online through the ProTOX tool. The chemical structures of tested pyrethroids (Type 1 and Type 2) were retrieved from PubChem database.

Protein preparation

The immune proteins crystal structures (1BQH-2.8 Å; 1GC1-2.5 Å; 1YGR-2.9 Å; 1YGR-2.7 Å) were obtained from RCSB Protein Bank. The crystal structure of CD8 (PDB: 1BQH) and CD4 (PDB: 1GC1) glycoprotein was reported to complex with NAG and NDG; NAG, NDG, and α-L-fucose, respectively. Another immune receptors such as CD45 and CD28 (PDB: 1YGR and 1YJD) were found in complex with MET, TYR, and NAG, respectively. Protein preprocess and ionization steps were executed to receptors molecule, a crucial step to the correct geometry of receptors.

Ligand library

The tested compounds preparations highlighting energy minima were completed using least square OPLS_2005 force field. The conformers were too generated and filtered to their energy minima with probable state creation at pH 7 ± 2.0.

Grid generation and docking calculation

The sitemap option was employed (immune proteins) to generate the possible binding site for hydrophobic, hydrophilic, and H-bond donor/acceptor regions. Extra precision (XP) Glide docking was employed and finally docking pose examined through XP Visualizer, indicating possible interactions of the tested entities with the diverse residues of immune receptors.

RESULTS AND DISCUSSION

The ranking of screened pyrethroid derivatives was evaluated by the binding energy of the ligands. Table 1 reveals that fenvalerate (−5.534 kcal/mol) possess a higher binding affinity with CD8 (1BQH) immune receptor while Tables 2 and 3 show a higher binding affinity for fluvalinate (−4.644 kcal/mol and −4.431 kcal/mol) against CD4 (1GC1) and CD45 (1YGR) immune receptor, correspondingly. Moreover, cypermethrin (−3.535 kcal/mol) showed greater glide score toward CD28 (1YJD) immune protein [Table 4]. The estimated free energy of binding should not be used as an only criterion for the selection of top hits but visual

Table 1: Glide score, number of hydrogen bonds, and residues involved in hydrogen bonding interaction of pyrethroids with immune receptor (PDB: 1 BQH)

Name of compound	Glide score	Numeral hydrogen bonding	Residue concerned with hydrogen bonding
Fenvalerate	−5.534	1	Asn70
Cypermethrin	−5.370	2	Asn70, Tyr159
Tralomethrin	−5.319	1	Tyr159
Flumethrin	−5.155	1	Lys66
Fluvalinate	−5.133	1	Arg155
Cyfluthrin	−5.021	1	Lys66
Permethrin	−4.952	1	Lys66
Fenpropathrin	−4.859	1	Asn70
Phenothrin	−4.823	1	Lys66
Flucythrinate	−4.780	1	Arg155
Deltamethrin	−4.670	1	Lys66
Resmethrin	−4.555	-	-
Tefluthrin	−4.325	1	Lys66
Bifenthrin	−4.100	-	-
Tetramethrin	−4.087	1	Gln114
Allethrin	−4.081	2	Asn70, lys66
Cyhalothrin	−3.583	-	-

Table 2: Glide score, number of hydrogen bonds, and residues involved in hydrogen bonding interaction of pyrethroids with immune receptor (PDB: 1GC1)

Name of compound	Glide score	Numeral hydrogen bonding	Residue concerned with hydrogen bonding
Fluvalinate	−4.644	3	Phe277, Thr236
Flumethrin	−3.968	1	Lys348
Permethrin	−3.745	1	Lys348
Tralomethrin	−3.727	1	Lys348
Fenvalerate	−3.673	1	Lys348
Phenothrin	−3.670	1	Lys348
Fenpropathrin	−3.662	1	Ser274
Cypermethrin	−3.556	1	Lys348
Resmethrin	−3.536	1	Lys348
Flucythrinate	−3.524	1	Lys348
Cyfluthrin	−3.342	1	Lys348
Allethrin	−3.212	1	Lys348
Cyhalothrin	−2.976	1	Lys348
Tetramethrin	−2.961	1	Lys348
Tefluthrin	−2.854	-	-
Deltamethrin	−2.825	1	Lys348
Tralomethrin	−3.727	1	Lys348

Table 3: Glide score, number of hydrogen bonds, and residues involved in hydrogen bonding interaction of pyrethroids with immune receptor (PDB: 1 YGR)

Name of compound	Glide score	Numeral hydrogen bonding	Residue concerned with hydrogen bonding
Fluvalinate	−4.431	2	Arg734, Arg657
Flucythrinate	−3.915	-	-
Fenvalerate	−3.831	1	Lys736
Resmethrin	−3.750	-	-
Permethrin	−3.714	1	Asp660
Cypermethrin	−3.554	1	Lys736
Flumethrin	−3.523	1	Arg734
Allethrin	−3.513	-	-
Fenpropathrin	−3.498	1	Lys736
Phenothrin	−3.422	-	-
Tetramethrin	−3.163	1	Lys736
Tefluthrin	−3.156	-	-
Deltamethrin	−3.056	-	-
Cyfluthrin	−2.716	-	-
Cyhalothrin	−2.712	1	Lys736
Bifenthrin	−2.702	-	-
Tralomethrin	−2.494	-	-

Table 4: Glide score, number of hydrogen bonds, and residues involved in hydrogen bonding interaction of pyrethroids with immune receptor (PDB: 1 YJD)

Name of compound	Glide score	Numeral hydrogen bonding	Residue concerned with hydrogen bonding
Cypermethrin	−3.535	2	Gln59, Asn53
Permethrin	−3.523	2	Gln59, Asn53
Flucythrinate	−3.462	2	Gln56, Asn53
Phenothrin	−3.440	2	Gln59, Asn53
Fenpropathrin	−3.195	2	Asn53, Gln56
Fluvalinate	−3.099	2	Gln56, Asn53
Fenvalerate	−3.059	2	Asn53, Gln59
Allethrin	−2.826	2	Gln56, Asn53
Resmethrin	−2.567	1	Gln56
Cyfluthrin	−2.161	1	Asn53
Tefluthrin	−2.027	-	-
Bifenethrin	−1.883	1	Ser55
Flumethrin	−1.869	1	Asn53
Deltamethrin	−1.741	-	-
Tralomethrin	−1.587	-	-
Cyhalothrin	−1.381	-	-
Tetramethrin	-	-	-

Table 5: Toxicity prediction of top-ranked glide score pyrethroids using ProTOX tool

Compounds	Toxicity class*	LD_{50} (mg/kg)	Possible toxicity targets (uniprot name)
Fenvalerate	3	70	AOFA and PGH 1
Fluvalinate	3	216	AOFA and PGH 1
Cypermethrin	2	25	AOFA and PGH 1

*Class 1: Fatal if swallowed (LD_{50} ≤5 mg/kg); Class 2: Fatal if swallowed (5 <LD_{50} ≤300 mg/kg); Class 3: Toxic if swallowed (50< LD_{50} ≤300 mg/kg); Class 4: Harmful if swallowed (300< LD_{50} ≤2000 mg/kg); Class 5: May be harmful if swallowed (2000< LD_{50} ≤5000 mg/kg); Class 6: Nontoxic (LD_{50} >5000 mg/kg). AOFA: Amine oxidase (flavin-containing) A; PGH 1: Prostaglandin G/H synthase 1

inspections of docking pose can too serve as an important predecessor for enhancing accomplishment of our docking screening results.[28] The best hit ligands were taken into the account for further toxicity prediction in rodents using ProTOX online tool. Table 5 results also illustrated that fenvalerate, fluvalinate, and cypermethrin have probable toxicity targets such as AOFA: Amine oxidase (flavin-containing) A and PGH1: Prostaglandin G/H synthase 1, respectively.

Apex-graded pyrethroid hits

CD8 (PDB: 1BQH) immune receptor

Fenvalerate

Fenvalerate has positioned the apex hit toward CD8 receptor as indicated in Table 1. This ligand was bound strongly to the binding site by nitrile group (CN) hydrogen bonding interactions with Asn70. Moreover, Tyr159, Leu156, Tyr45, Tyr7, Tyr22, Val9, Val97, Tyr116, and Phe74 were concerned in the hydrophobic interactions. It also showed two π–π stacking interactions with Tyr7 and Tyr159.

Cypermethrin

Cypermethrin has engaged hydrophobic in pocket of Phe74, Val97, Val9, Tyr116, Tyr22, Tyr7, Tyr159, Leu5, Trp167, Cys164, Tyr167, Tyr59, and Tyr45, and moreover, two hydrogen bonds between Asn70 with CN group and Trp159 with the oxygen of phenyl-linked ring are also studied. Interestingly, ligand phenyl ring illustrated π–cation, π–π interactions with Lys66 and Trp167.

Tralomethrin

The hydrophobic surface was occupied via Leu156, Val97, Tyr22, Val9, Phe74, Tyr159, Tyr7, Tyr167, Leu5, Tyr171, Cys164, Tyr59, and Tyr45 residues. Furthermore, Tyr159 had involved in hydrogen bonding interaction while π–cation (Lys166) and π–π interactions (Trp167) were also observed.

CD4 (PDB: 1GC1) immune receptor

Fluvalinate

Overall, fluvalinate resulted in the top most capable hit with a CD4 immune receptor. This compound has revealed promising hydrogen bonding of CN and NH groups with Thr236 while oxygen (other phenyl ring linked) moiety against Phe277. The hydrophobic interactions were also examined (Val275, Trp96, Phe233, Cys239, Pro238, and Phe277).

Flumethrin

The compound was capable of wide-ranging hydrophobic bonding with Ile232, Phe353, Val271, Pro238, Phe233, Phe277, Cys239, Val275, and Trp96. Conspicuously, one hydrogen bond of CN group with Lys348 and π–π stacking with Phe277 was also analyzed.

Tralomethrin

Tralomethrin was linked with the binding pocket via hydrogen bonding interactions of CN and carbonyl group with Lys348. Interestingly, Val271, Phe353, Ile272, Phe277, Phe233, Pro238, and Cys239 were involved in hydrophobic interactions while π–π interaction was also seen.

CD45 (PDB: 1YGR) immune receptor

Fluvalinate

This compound was resulted as top strike and involved in hydrophobic interaction with Tyr658, Val659, Val832, Ile661, Leu869, and Ala830 along with hydrogen bonding interactions with Arg657 (-NH group) and Arg734(C=O group).

Flucythrinate

This ranked entity was minimally able of hydrophobic interactions such as Ile661, Tyr658, Phe632, Val873, Ala830, Leu867, and Val832 with a CD45 immune receptor.

Fenvalerate

Fenvalerate was capable of H-bonding interaction of CN group with Lys736 residue. In addition, Val832, Ala830, Leu869, Tyr658, and Ile661 residues were also occupied in hydrophobic interactions.

CD28 (PDB: 1YJD) immune receptor

Cypermethrin

This apex hit compound has revealed two hydrogen bonding interactions with Gln59 (C=O group) and Asn53 (oxygen-phenyl linked).

Permethrin

The different amino acid residues such as Tyr61, Tyr51, Tyr54, and Tyr100 had resulted in hydrophobic interactions while C=O group and oxygen (phenyl ring linked) were too involved in hydrogen bonding interactions with Gln59 and Asn53, respectively. Conspicuously, a π–π interaction with Tyr54 was too seen.

Flucythrinate

This compound was an outcome in good hydrogen bonding interactions of C=O group and oxygen (phenyl ring linked) with Asn53 and Gln56, consequently. Moreover, flucythrinate showed hydrophobic interactions with different receptor residues such as Tyr51, Tyr54, and Tyr100 [Figures 1-4].

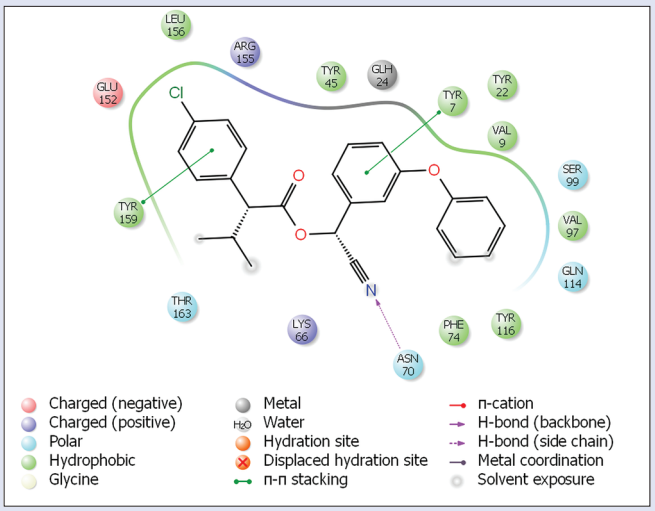

Figure 1: Binding interactions of Type 2 pyrethroid (fenvalerate) with CD8 immune cell receptor (PDB: 1BQH)

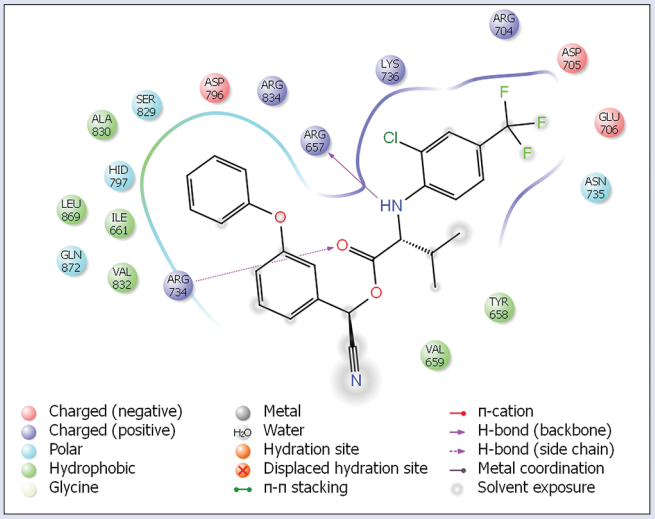

Figure 3: Binding interactions of Type 2 pyrethroid (fluvalinate) with CD45 immune cell receptor (PDB: 1YGR)

Consequences of the interaction of pyrethroid insecticides toward immune cell receptor

Remarkably, due to high sensitivity, the immune system is most easily concern with the toxicity of pyrethroids. Previous studies have shown that any alteration in the immune system serves as a vital predecessor for making an individual immunocompromised and more susceptible to serious health hazards.[29] The current findings have revealed that Type 2 pyrethroids exposed good interactions with immune cell proteins which may be linked to different pathways such as no alteration, autoimmune diseases, declined in the immune response, and development of hypersensitivity reactions [Figure 5].

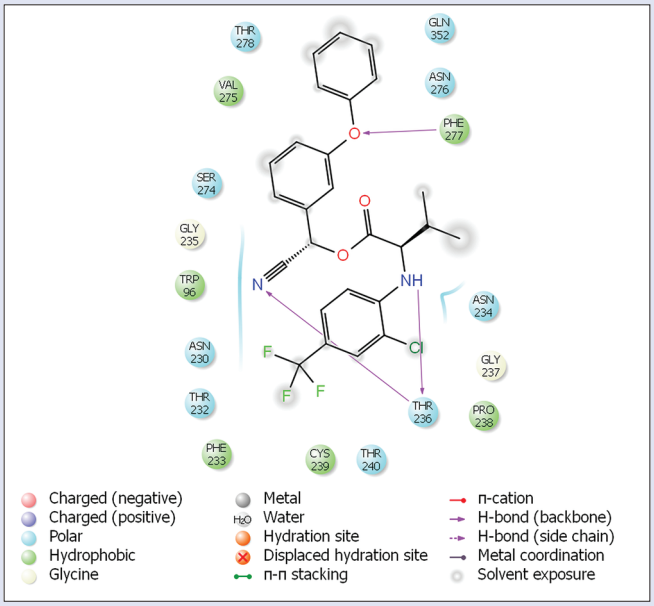

Figure 2: Binding interactions of Type 2 pyrethroid (fluvalinate) with CD4 immune cell receptor (PDB: 1GC1)

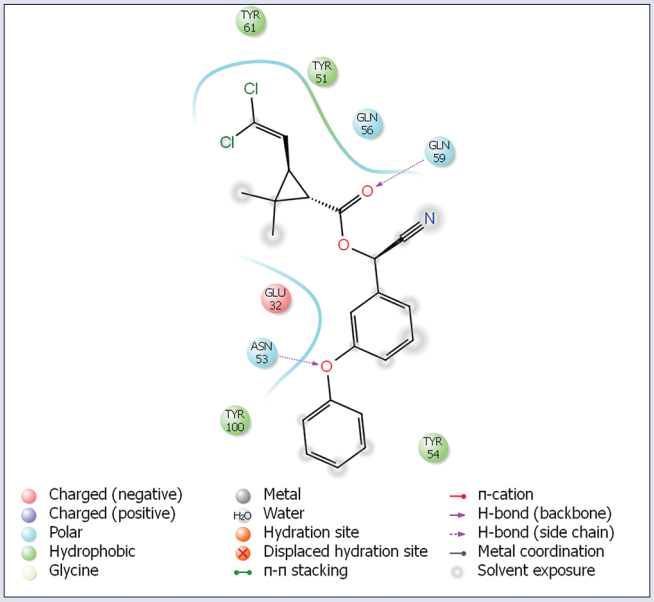

Figure 4: Binding interactions of Type 2 pyrethroid (cypermethrin) with CD28 immune cell receptor (PDB: 1YJD)

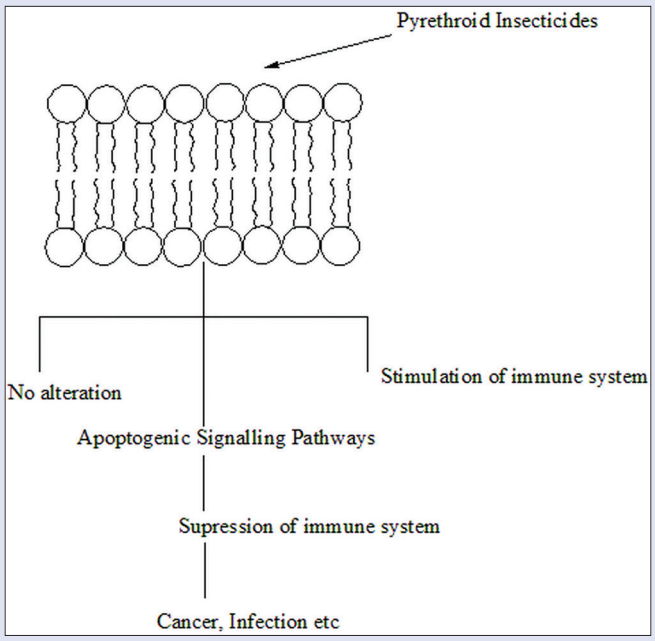

Figure 5: Probable immune reactions of pyrethroids with immune receptors

CONCLUSION

Type 2 pyrethroids such as fenvalerate (1BQH), fluvalinate (1GC1 and 1YGR), and cypermethrin (1YJD) have outcome in apex-graded immunotoxicity ligands. Interestingly, toxicity of top-ranked docked pyrethroids has also been analyzed with LD_{50} value plus possible toxic targets. Although pyrethroids have become popular due to their promising applications in different fields, current *in-silico* immunotoxic assessments of type 2 pyrethroids have put a big question mark pertaining to human health issues. This tool may further quite helpful for future researchers to validate the results with wet laboratory experiments.

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Conflicts of interest

There are no conflicts of interest.

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