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.

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-phenoxyl-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...
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. Pyrethroid insecticides are a product of choice in many countries due to their expeditious metabolism rate and low toxicity to humans and other non-target 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. 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. 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. 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. 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 being. Immunoxicity 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 LD₅₀ value against rodents. 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 (Schrodinger, 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 preprocessing 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

| Name of compound | Glide score | Numeral hydrogen bonding | Residue concerned with hydrogen bonding |
|------------------|-------------|--------------------------|----------------------------------------|
| Fenvalerate      | −5.534      | 1                        | Asn70                                  |
| Cypermethrin     | −5.370      | 2                        | Asn70, Tyr159                          |
| Tefluthrin       | −3.19       | 1                        | Tyr159                                 |
| Flumethrin       | −5.155      | 1                        | Lys66                                  |
| Fluvinate        | −5.133      | 1                        | Arg155                                 |
| Cyfluthrin       | −5.021      | 1                        | Lys66                                  |
| Permethrin       | −4.952      | 1                        | Lys66                                  |
| Fenpropathrin    | −4.859      | 1                        | Asn70                                  |
| Phenothenin      | −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      | -                        | -                                      |

| Name of compound | Glide score | Numeral hydrogen bonding | Residue concerned with hydrogen bonding |
|------------------|-------------|--------------------------|----------------------------------------|
| Fluvinate        | −4.644      | 3                        | Phε777, Thr236                          |
| Flumethrin       | −3.968      | 1                        | Lys348                                 |
| Permethrin       | −3.745      | 1                        | Lys348                                 |
| Tefluthrin       | −3.727      | 1                        | Lys348                                 |
| Fenvalerate      | −3.673      | 1                        | Lys348                                 |
| Phenothenin      | −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 |
|------------------|-------------|--------------------------|----------------------------------------|
| Fenvalerate      | −3.353      | 2                        | Gln59, Asn53                            |
| Permethrin       | −3.323      | 2                        | Gln59, Asn53                            |
| Fluvalinate      | −3.462      | 2                        | Gln56, Asn53                            |
| Phenothrin       | −3.440      | 2                        | Gln59, Asn53                            |
| Fenpropathrin    | −3.195      | 2                        | Gln53, Gln56                            |
| Fluvalinate      | −3.099      | 2                        | Gln56, Asn53                            |
| Fenvalerate      | −3.059      | 2                        | Gln53, Gln59                            |
| Allethrin        | −2.826      | 2                        | Gln56, Asn53                            |
| Resmethrin       | −2.567      | 1                        | Gln56                                  |
| Cyfluthrin       | −2.161      | 1                        | Asn53                                  |
| Tefluthrin       | −2.027      | -                        | -                                      |
| Bifenthrin       | −1.883      | 1                        | Ser55                                  |
| Tralomethrin     | −1.741      | -                        | -                                      |
| Tralomethrin     | −1.587      | -                        | -                                      |
| Cyhalothrin      | −1.381      | -                        | -                                      |
| Tetramethrin     | -           | -                        | -                                      |

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

| Name of compound | Glide score | Numeral hydrogen bonding | Residue concerned with hydrogen bonding |
|------------------|-------------|--------------------------|----------------------------------------|
| Cypermethrin     | −3.335      | 2                        | Gln59, Asn53                            |
| Permethrin       | −3.323      | 2                        | Gln59, Asn53                            |
| Fluvalinate      | −3.462      | 2                        | Gln56, Asn53                            |
| Phenothrin       | −3.440      | 2                        | Gln59, Asn53                            |
| Fenpropathrin    | −3.195      | 2                        | Gln53, Gln56                            |
| Fluvalinate      | −3.099      | 2                        | Gln56, Asn53                            |
| Fenvalerate      | −3.059      | 2                        | Gln53, Gln59                            |
| Allethrin        | −2.826      | 2                        | Gln56, Asn53                            |
| Resmethrin       | −2.567      | 1                        | Gln56                                  |
| Cyfluthrin       | −2.161      | 1                        | Asn53                                  |
| Tefluthrin       | −2.027      | -                        | -                                      |
| Bifenthrin       | −1.883      | 1                        | Ser55                                  |
| Tralomethrin     | −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       | Class 3         | 70              | AOFA and PGH 1                          |
| Fluvalinate       | Class 3         | 216             | AOFA and PGH 1                          |
| Cypermethrin      | Class 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 and PGH1: 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.
CD28 (PDB: 1YJD) immune receptor

Cypemethrin

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].

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. 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].
CONCLUSION

Type 2 pyrethroids such as fenvalerate (1BQH), fluvanlate (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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