New Hit Compounds Targeting Odorant Binding Proteins (OBPs) as Putative Repellents

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1. Introduction

Mosquitoes and other hematophagous arthropods, the primary vectors of multiple parasites and viruses, are responsible for the transmission of serious and potentially fatal diseases to humans, such as malaria, dengue fever, West Nile fever, leishmaniasis and more recently Zika virus[1] infection. According to the World Health Organization, the prevalence of human mortality causing from infected mosquitoes amounts to one million, annually.[2,3]

Nowadays, the increasing resistance of vectors to existing repellents render them as ineffective, creating the need for development novel repellents with advanced properties to the existing ones in terms of duration of the protection, minimum effective dose, efficacy against a wide variety of insects' bites and safety.

The main goal of the current study is the discovery of novel hit compounds which may evolve as insect repellents by performing a combination of computational and analytical chemistry methodologies. Particularly, a pharmacophore-based virtual screening of natural compound libraries coupled with molecular docking were applied to identify novel hits towards Odorant Binding Protein 1 (OBP1) a molecular target for the most widely used synthetic repellents DEET[4,5] and Icaridin[6]. Compounds were selected to bear the appropriate physicochemical properties associated with insect repellency. Six compounds were evaluated against female mosquitoes (*Aedes albopictus)*. Results presented insect repellent activity of 35-57,9% compared to untreated hand which indicate the proposed scaffolds as starting points for further structure optimization.

2. Pharmacophore Model Generation

A focused virtual combinatorial library was created using CombiGlide module of MAESTRO. This library, consisting on 6628 structures, was subjected to further molecular docking studies to *Agam*OBP1.[3]

In addition to binding affinities and the other quantitative energetic criteria, the selection of the most remarkable compounds of the virtual library was based on the presence of interactions with crucial amino acids. Specifically, the main residues participating in the binding site interactions with insect repellents of OBP1 are His77, Ala88, Trp114, Leu76, Leu73, Leu80 and Met91.

By using the above-mentioned criteria, 46 visual structures bearing different scaffolds and structural features were selected for the creation of structure-based pharmacophore models. The pharmacophore features of all these models were aligned and an optimum pharmacophore model including only common features was generated (Fig. 2).

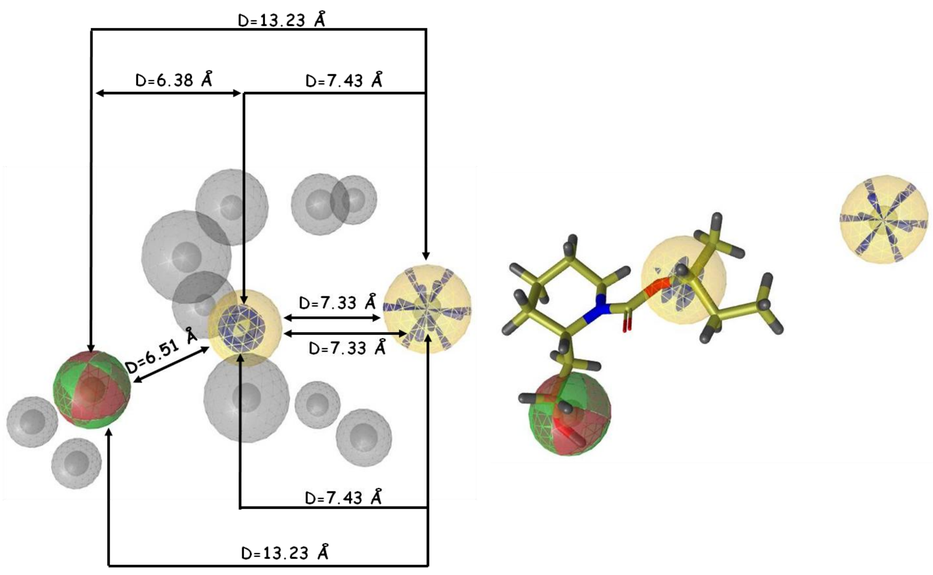

Figure 2: Features of the optimum structure-based pharmacophore model (left) and Icaridin fitted on the final pharmacophore model (right), The features are depicted with the following color coding: hydrogen bond acceptor (HBA) as red sphere, hydrogen bond donor (HBD) as green sphere, hydrophobic regions (H) as yellow spheres, aromatic ring (AR) as blue ring, positive ionizable region (NIR) as blue stick and exclusion volumes (Ex. Vol.) as grey spheres. The distances (Å) between the chemical features are illustrated as black lines.

This model comprised of six features and more specifically one hydrogen bond acceptor (HBA), one hydrogen bond donor (HBD), two hydrophobic regions (H),one aromatic ring (AR) and one positive ionizable region (NIR) (Fig. 2). The pharmacophore model was finally optimized by changing the number and the size of the exclusion volumes (10 Ex. Vol.) according to the alignment of the structures of the virtual combinatorial library.

The validity of the model was confirmed by the fact that Icaridin (Fig. 2) fitted optimal to four features of the final pharmacophore model (Pharmacophore fit score: 46.37).

3. Pharmacophore-based virtual screening

The Sigma-Aldrich (https://www.sigmaaldrich.com/) database, containing ~ 67000 synthetic compounds and the Chemical Database of Traditional Chinese Medicine, including ~250000 natural products were subjected to pharmacophore-based virtual screening. Hits with the top ranked pharmacophore fit score (44-67) were further docked at the binding site of *Agam*OBP1. Finally, 6 compounds (Fig. 3) were selected for further *in vivo* behavioral studies against female mosquitoes and the docked poses of each of selected compounds are illustrated in Figure 4.

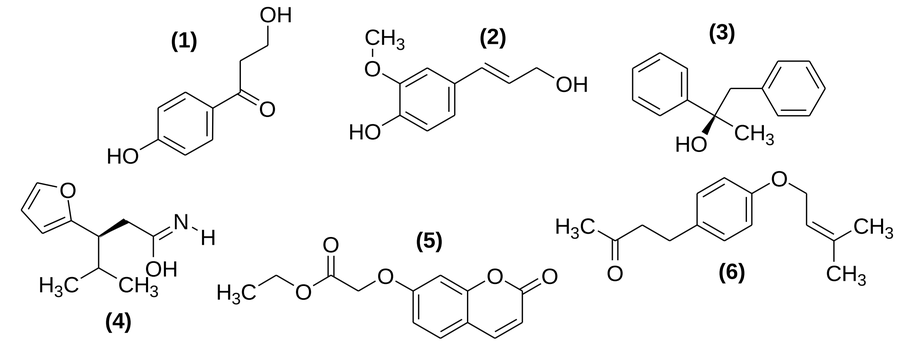

Figure 3. Shortlist of selected compounds.

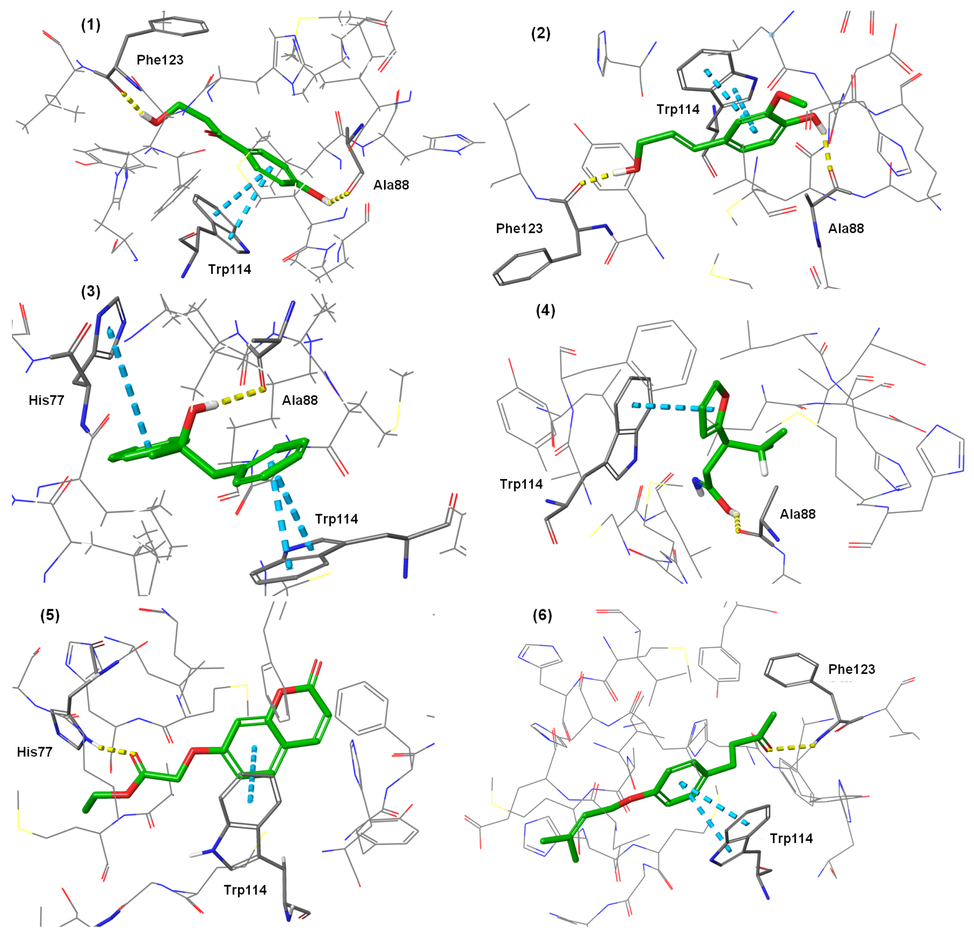

Figure 4. Binding poses of the selected compounds (1-6) from molecular docking studies. All six compounds are presented in green sticks and *Agam*OBP1 residues in grey. The hydrogen bonds and the π-πstacking interactions are illustrated with yellow and blue dashed lines, respectively.

4. *In vivo* behavioral assays

All examined compounds exhibited insect repellent activity (35-57.9%), against female mosquitoes (*Aedes albopictus*), compared to naked hand. In specific, the % insect repellency of compounds 1, 2, 3, 4, 5 and 6, compared to untreated hand, is 57.9%, 48.4%, 48.4%, 42.6%, 38.8% and 35%.

Interestingly, compound 1 presents the most remarkable repellent activity.

5. Conclusions

- ❑ Virtual screening was implemented to identify compounds which can be employed as insect repellents bearing new scaffolds than commercial available products.
- ❑ All six selected compounds were identified as potent insect repellents with the most potent one (**4**) to exhibit 57.9% repellency in comparison to untreated hand, serving an ideal starting point for hit to lead optimization process.

6. References

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