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In Silico Evaluation of Synthetic Hydrophobic Fluorescent NBD- and DANSYL-Derivatives as Potential Inhibitors of Insect Chitinases †

Yaroslav Faletrov [1,2], Polina Yakovets [1,2] and Nina S. Frolova [2,*]

[1] Faculty of Chemistry, Belarusian State University, Leningradskaya Str. 14, Minsk, Belarus; yaroslav82@tut.by (Y.F.); email@email.com (P.Y.)
[2] Research Institute for Physical Chemical Problems, Belarusian State University, Minsk, Belarus
* Correspondence: email@email.com
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Abstract

Docking calculations in semi-automatic virtual screening mode have been done using AutoDock Vina (5 × 5 × 5 nm grid box, centered on the chain A) and FYTdock helper software. N-hexanoyl ciprofloxacin has been found to bind with chitinases from *Ostrinia furnacalis* (pdb : 7vrg, 6jaw, 6jay, 6jmn, 5y2b; energy of bindings (Ebind) -10.2…-9.7). N-hexanoyl-N′-NBD-piperazine, NpipHex, bind in silico with the enzyme less effectively (pdb codes: 6jaw, 5y2b, 6jay, 5y2c, 3wkz; Ebind -9.3…-8.9). Lipid-like N-NBD-oleylamine and N-Dansyl-oleylamine demonstrated quite similar, but smaller affinity (Ebind -8.6…-8.0). Examples of interactions close to the active sites of the chitinases were found for all compounds. These results provide new insights into insect biochemistry of chitinases showing new molecular scaffolds suitable as prototypes of tools for pest control or fluorescence-based screening.

Keywords: fluorescent compounds; chitinase; docking

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1. Introduction

Chitinases (EC 3.2.1.14, 1,4-beta-poly-N-acetylglucosaminidases) are enzymes catalyzing hydrolytic cleavage of glyosidic β-1→4-bonds in chitin (poly(β-(1→4)-N-acetyl-d-glucosamine). They are found in plants [1], yeast and fungi [2], bacteria [3], algae, crustaceans and insects [4,5] as well as in humans [6] and other organisms. In insects, including pests and diseases vectors, the enzymes play important roles during ecdysis and, for some species, feeding. Thus, insect chitinases are promising as molecular targets for new insecticides development aiming crop protection and healthcare purposes.

A number of 3D structures for insect chitinases have been solved and presented in Protein data Bank (https://www.rcsb.org/) online repository, e.g. from moth Asia corn borer *Ostrinia furnacalis*. The circumstance opens a good option to use in silico approaches to select possible new affine ligands of the enzymes [7, 8] from vast number of compounds in chemical space to rationalize further in vitro tests. Notably, some chitinases have a lot of hydrophobic amino acids residues close to catalytic sites, resulting in a selection criterion for possible inhibitors. Using the circumstance, a fact about fatty acid blocks in structures of novel azamacrolide chitinase inhibitor [9] and the repurposing approach we

decided to evaluate *in silico* few lipophilic molecules from our lab, including two previously reported fluorescent N-hexanoyl-piperazine derivatives based on 7-nitrobenzofurazan-4-yl- (NBD-) and ciprofloxacin (CPF) in aspects of cytochromes P450 inhibition and biocompatible polymerization photo-initiator [10, 11]. In this work we provide an insight that such compounds cold be prototypes for insect chitinase inhibitors.

2. Materials and Methods

The chosen structures for docking were as follow: N-hexanoyl-ciprofloxacin (CPF-Hex), N-hexanoyl-N′-(NBD-)piperazine (NpipHex), N-hexanoyl-N′-(NBD-)ethylenediamine (NedaHex), N-NBD-oleylamine (NOLA) and N-dansyl-oleylamine (DOLA). Their structures are depicted on Figure 1.

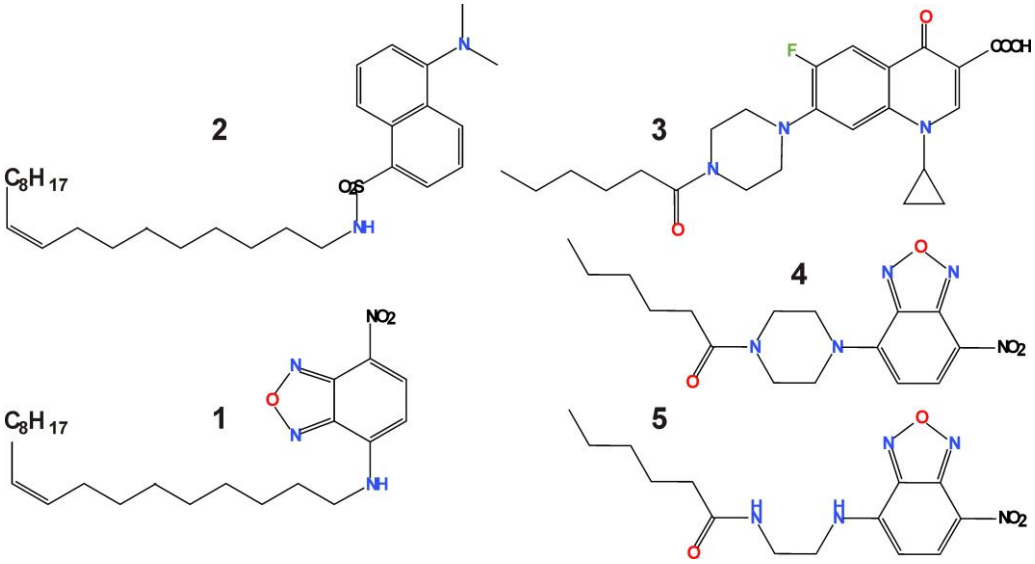

Figure 1. Chemical structures of the compounds under consideration. 1—NOLA, 2—DOLA, 3—CPFHex, 4—NpipHex, 5—NedaHex.

Docking calculations in semi-automatic virtual screening mode have been done using AutoDock Vina v. 1.1.2 and FYTdock helper software based on Python scripts and Microsoft Excel tables [12] to organize, run and analyze results. Chains A only, 5 × 5 × 5 nm grid box (centered on a protein), exhaustiveness of 15 were used. The PDB codes for 3D structures of proteins from *Ostrinia furnacalis* used were as follow: 3wmc, 9g3q, 3wmb, 7vrg, 5gpr, 3wqw, 3wl1, 3wqv, 5wv9, 5y2a, 5y2c, 5wvb, 5wus, 5wvg, 5wvf, 5wvh, 5gqb, 6jmn, 3w4r, 6jax, 6jay, 5wup, 5wv8, 3wkz, 3wl0, 6jav, 5y2b, 5y29, 6jaw, 6jmb, 6jm8, 6jm7. Values for docking scores (energies of binding, Ebind, kcal/mol) and amino acid surroundings predicted for the compounds were tabulated. Only top-5 of docking simulations results based on Ebind values are mentioned in the article. Figures were prepared using Biovia Discovery Studio software v. 16.1.0.

3. Results

Results of docking simulations of aforesaid chitinase and compounds are given in Tables 1–5.

Table 1. Top 5 of docking simulations results of interactions of insect chitinase with CPFHex.

PDB Code	Ebind	Amino Acids Surrounding [1]
7vrg	−10.2	Ser357, Gly359, Lys362, Tyr411, Ala358, Phe309, Met381, Asp384, Glu308, *Trp268*, *Trp389*, Arg439, *Trp532*, Tyr437, Val469, Glu533, Phe385
6jav	−9.9	Val1740, Asn1778, ***Trp1809***, ***Trp1691***, Asp1804, Pro1777, Tyr1803, Tyr1734, Gln1858, Phe1899, ***Trp1961***, Tyr1856, Asp1731, ***Glu1733***, Met1801
6jaw	−9.9	Val1740, Asn1778, ***Trp1809***, ***Trp1691***, Asp1804, Tyr1734, Gln1858, Tyr1856, Phe1899, ***Trp1961***, Tyr1803, ***Glu1733***, Asp1731
6jay	−9.9	***Trp1809***, Val1740, ***Trp1691***, Asp1804, Tyr1734, Gln1858, Tyr1856, Phe1899, ***Trp1961***, Tyr1803, ***Glu1733***, Phe1648
6jmn	−9.9	Ser357, Gly359, Lys362, Tyr411, Ala358, Phe309, Asp384, Met381, *Trp268*, Glu308, *Trp389*, Arg439, *Trp532*, *Trp160*, Phe184, Thr269, Phe385

[1] The active site residues are typed using *italic*.

Table 2. Top 5 of docking simulations results of interactions of insect chitinase with NpipHex [1].

PDB Code	Ebind	Amino Acids Surrounding
6jaw	−9.3	Trp1621, ***Trp1961***, Phe1899, Ala1896, Arg1625, Gln1858, Asp1804, ***Trp1691***, ***Trp1809***
5y2b	−9.1	Phe1648, Asn1692, Asp1693, Gly1689, Gly1690, ***Trp1691***, ***Trp1621***, His1660, ***Trp1961***, Asp1804, Gln1858, Tyr1856, Phe1899, Leu1965
6jay	−9.1	Trp1621, ***Trp1961***, Phe1899, Ala1896, Arg1625, Thr1894, Gln1858, Asp1804, ***Trp1691***, ***Trp1809***, Val1740
5y2c	−9	Phe2094, Gly2136, Asp2139, Asp2140, Gly2137, Trp2138, Trp2067, His2107, Trp2398, Phe2336, Tyr2303, Gln2305, Leu2402
3wkz	−8.9	Tyr217, Tyr272, Trp372, Asp218, Gln148, Trp107, Phe61, Met215, Asp146, Trp34, Phe309, Arg274, Ala306, Arg38, Thr304

[1] The active site residues are typed using *italic*.

Table 3. Top 5 of docking simulations results of interactions of insect chitinase with NedaHex.

PDB Code	Ebind	Amino Acids Surrounding [1]
5gpr	−9	*Trp268*, Asp384, Arg439, Phe309, Tyr383, Ala358, Met381, Ser357, Gly359, Lys362, Phe385, Tyr411, *Glu308*, *Trp532*, Phe184, Tyr156, *Asp306*, Ala355
6jmn	−9	*Trp268*, Asp384, Arg439, Phe309, Met381, Ala358, Tyr383, Ser357, Gly359, Lys362, Tyr411, *Glu308*, *Trp532*, Tyr437, Tyr156, Phe184, *Asp306*
7vrg	−9	Met381, Tyr383, Asp384, Ala358, Phe309, Lys362, Ser357, Tyr411, Gly359, *Glu308*, *Trp268*, Arg439, *Trp389*
3nsn [2]	−9	Glu328, Trp483, Trp490, Glu526, Val327, Val484, Asn489, Arg220, Trp524, Tyr475, Glu368, Trp448, Asp367, Trp424
3ozp [2]	−8.9	Glu328, Trp483, Trp490, Glu526, Val327, Asn489, Asp477, Arg220, Trp524, Glu368, Asp367, Trp448, Tyr475, Trp424

[1] The active site residues are typed using *italic*. [2] The structures are for OfHex1 enzyme.

Table 4. Top 5 of docking simulations results of interactions of insect chitinase with NOLA.

PDB Code	Ebind	Amino Acids Surrounding [1]
6jmn	−8.6	Lys362, Tyr411, Ser357, Gly359, Ala358, Phe309, Asp384, *Trp268*, *Trp389*, Arg439, *Glu308*, Phe184, *Trp532*, Tyr383, Tyr156, Met381, *Asp306*
6jm8	−8.4	Trp33, Arg37, Tyr303, Trp365, Glu368, Ser366, Tyr270, Glu300, Phe60, Trp105, Ser106, Tyr213, Asp214, Met211, Arg272, Trp219, Thr369
5gqb	−8.2	Ser357, Ala358, Gly359, Tyr411, Phe385, Met381, Tyr383, Phe309, Asp384, Lys362, *Trp389*, *Trp268*, Arg439, *Trp532*, Phe184, *Glu308*, *Asp306*, Tyr156
7vrg	−8.1	Ser357, Ala358, Gly359, Lys362, Tyr411, Met381, Tyr383, Phe309, *Trp389*, *Trp268*, Asp384, Arg439, *Glu308*, Phe184, *Trp532*
6jmb	−8	Tyr147, Arg150, Ser186, Val188, Trp219, Asp214, Trp105, Arg272, Phe60, Trp365, Glu146, Met211, Tyr213, Tyr270, Ala187, Ala191, Met215

[1] The active site residues are typed using *italic*.

Table 5. Top 5 of docking simulations results of interactions of insect chitinase with DOLA.

PDB Code	Ebind	Amino Acids Surrounding [1]
6jav	−8.7	***Glu1733***, Met1801, Tyr1734, Tyr1803, Asp1804, Val1740, Pro1777, Asn1778, ***Trp1809***, ***Trp1691***, Asp1731, ***Trp1961***, Phe1648, ***Trp1621***, Phe1899
5y2b	−8.5	***Trp1809***, Gln1858, Phe1899, Tyr1856, Asp1804, ***Trp1961***, Leu1965, ***Trp1621***, ***Trp1691***, Tyr1803, Phe1648, ***Glu1733***, Tyr1734, Val1740
7vrg	−8.5	*Trp268*, Arg439, *Trp532*, Phe184, *Glu308*, Tyr383, Asp384, Met381, Phe309, Ala358, *Trp389*, Lys362
3wqv	−8.4	Trp34, Phe61, Trp107, Trp372, Glu148, Tyr217, Tyr272, Asp218, Arg274, Phe309, Ala306, Arg38, Thr304, Ala108, Gly106, Glu109, Ile74, Asn33
5gpr	−8.4	Asp384, *Trp389*, Phe309, *Trp268*, *Glu308*, Arg439, *Trp532*, Phe184, Tyr383, Tyr156, *Asp306*, Ala355, Met381, Lys362

[1] The active site residues are typed using *italic*.

Localization details of the most affine interaction in the set is shown on Figure 2.

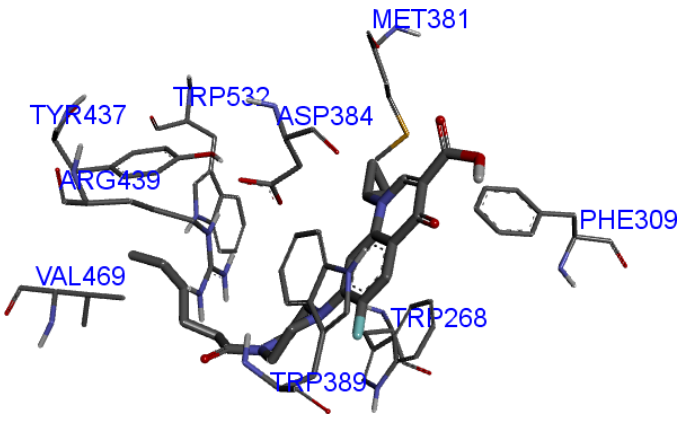

Figure 2. Calculated geometry of localization and amino acid surrounding of CPFhex close to the active site of chitinase from O. *furnacalis* (PDB 7vrg) with predicted Ebind of −10.2.

4. Discussion

The new in silico data concerning potential affinities of aforementioned fluorescent hydrophobic compound to chitinases of *O. furnacalis* was reported for the first time. The best affinity (the lowest Ebind) was obtained for CPFHex, an acylated derivative of the well-known FDA-approved antibacterial compound ciprofloxacin, and a structure of *Of*ChiH (PDB 7vrg). The active site of the chitinase include aa acidic catalytic triad (Asp304, Asp306, and Glu308) of DXDXE motif as well as substrate-binding cavity built of solvent-exposed aromatic residues of domain I (Trp27 and Trp63) and domain II (Trp160, Tyr163, Trp225, Trp238, Trp268, Trp389, and Trp532) [13]. In the predicted complex (Table 1) CPFHex was found to be surrounded at set of residues, including Glu308, Trp268, Trp389, Trp532, showing a partial overlap with the active site. Similarly, for othe chitinase structures equivalent residues are Glu1733, Asp1729 as well as Trp1621, Tyr1624, Trp1663, Trp1691, Trp1809, and Trp1961 [14]. As it can be seen from Table 1 - 5 all ligands tested demonstrate at least partial overlap with catalytic and substrate-binding centers of the chitinases structures.

It should be noted that till the publication, to the best of our knowledge, such compounds were not estimated as possible inhibitors of chitinases. Else, we found only one publication concerning a fluorescent substrate for Acidic mammalian chitinase (AMCase) based on fluoresceinated chitin oligomers [15] and the very recent one concerning NBD-based C-glycoside as fluorescent inhibitor of insect chitinases OfChtII and OfHex1 acting as insecticides [16]. In broader way, it can be noted that there is a lack of data concerning NBD-, DANSYL-, fluoroquinolone and other common fluorophore derivatives with chitinases of both mammals and insects in spite of potential of such derivatives as molecular probes and screening tools for the enzymes and growing interest to the proteins as potential targets for drugs [17,18] or new type insecticides [8]. These circumstances seem to highlight novelty of our virtual screening data and could encourage researchers to create new molecular tools, drugs or insecticides.

Notably, because pros and cons of ciprofloxacin for human health are considered to be studied well, itself and some its derivatives seem to be promising as a template of safe insecticides. From the other hand, our NBD-based and DANSYL-based structures represent original ones and, thus, their biosafety level is unknown, but during broad using of the fluorophores in various biophysical and biochemical researches also provide a chance to tune selective toxicity against insects. Also, notably, that there are a small number of reports concerning fluorescent compounds interactions with insect proteins, even at in silico level, and cell, in comparison with fluorescent probes usage for mammalian cells, yeasts and bacteria.

Our virtual screen was done in inverse mode using multiple chitinases structures. Such approach is good because it allows to take into account various differences of crystal structures of the same proteins providing a base for more weighted prognosis in spite of rigid docking limitations. Our helper software FYTdock is designed for such inverse virtual screening mode also provide an option for broader searches to discover new affine both off-target interactions and new plausible targets, like in this report. It is interesting to note that due to availability of recently developed novel technology Alphafold [19] model structures of chitinases for other insects can be obtained and taken in virtual screening mode to get insight on possible success or failure of future *in vitro* tests.

5. Conclusions

Here we report about virtual screening results which has involved 30 PDB chitinase structures and some original hydrophobic fluorescent compounds with 7-nitrobenzoxadiazol (NBD) and dansyl scaffolds. N-hexanoyl ciprofloxacin has been found to bind with

chitinases from *Ostrinia furnacalis* (the best result with Ebind -10.2). N-hexanoyl-N′-NBD-piperazine, NpipHex, its open-ring analogue of NpipHex, N-hexanoyl-N′-NBD-ethylenediamine, has demonstrated quite similar affinity. Also lipid-like N-NBD-oleylamine and N-Dansyl-oleylamine demonstrated less affine binding affinity. These results open opportunity for the corresponding in vitro tests with insects or purified chitinases as well as for wider in silico screen using Alphafold options to predict 3D structural models for others insect chitinases as well as other chitinases from various species to address healthcare and agricultural challenges.

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Abbreviations

The following abbreviations are used in this manuscript:

CPFHex	N-hexanoyl ciprofloxacin
NpipHex	N-hexanoyl-N′-(7-nitrobenzofurazan-4-yl-)-piperazine
NedaHex	N-hexanoyl-N′-(7-nitrobenzofurazan-4-yl-)-ethylenediamine
NOLA	N-(7-nitrobenzofurazan-4-yl-)-oleylamine
DOLA	N-Dansyl-oleylamine or N-(5-(Dimethylamino)naphthalene-1-sulfonyl)-oleylamine
Ebind	Energy of binding (docking score)

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