MOLECULAR DOCKING ANALYSIS OF NEWLY SYNTHESIZED 2-MORPHOLINOQUINOLINE DERIVATIVES WITH ANTIFUNGAL POTENTIAL TOWARD Aspergillus fumigatus

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The present paper is concerned with the molecular docking analysis of newly synthesized 2-morpholinoquinoline derivatives with antifungal potential toward Aspergillus fumigatus. The purpose of the molecular docking analysis was to determine potential interactions between the analyzed compounds and the active site of the enzyme glucosamine-6-phosphate synthase, as well as to reveal which molecular features (the presence of different substituents or isomers) could be responsible for significant antifungal activity of the tested compounds. The compounds with the highest antifungal potential toward pathogenic and saprotrophic fungus Aspergillus fumigatus were docked, and the results were compared with the docking of griseofulvin, which is an antifungal drug used in the treatment of various types of dermatophytoses. Newly discovered antifungal agents are very important in medicine, as well as in agriculture. The prevention of the presence of mycotoxins in food and feed products is one of the urgent tasks. Therefore, every effort which leads to discovery of their mechanism of action and determination of side effects is precious. The present study can be considered a contribution to the analysis and selection of newly discovered antifungals from the 2-morpholinoquinoline family, and one step forward to their practical use in medicine and agriculture. The obtained results reveal the possible reason why the newly synthesized 2-morpholinoquinoline expresses even higher antifungal activity toward Aspergillus fumigatus than griseofulvin, a commercially available antifungal therapeutic.

KEY WORDS: antifungal activity, Aspergillus fumigatus, fungi, molecular docking, molecular modeling.

INTRODUCTION

The presence of mycotoxins in food and feed products has become a global problem. These secondary metabolites of different fungi (usually from the genera Aspergillus, Penicillium and Fusarium) have an enormous influence on human health and agriculture. Aspergillus fumigatus is a fungus often isolated from decomposed plant material. Also, it is isolated from cereals, including wheat, rice, barley, soybeans and beans, and also from

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meat products, milk, and dairy products (1). The optimal temperature range for the growth of this fungus is between 40 and 42 °C (1). Fumitoxin, helvolic acid, fumagillin, gliotoxin, fumitremorgins, monotryptacinid, fumigaclavines, fumigatin, tryptoquivaline are the secondary metabolites commonly found in *Aspergillus fumigatus* (2). Some of the toxins produced by this fungus have immunosuppressive features. They can induce apoptosis in particular cells of the immune system. *Aspergillus fumigatus* has become one of the most important airborne pathogens in developed countries (2). It can cause some allergic reactions in humans as well (1). Due to high toxicity of the metabolites produced by *Aspergillus fumigatus*, it is highly important to prevent its presence in food and feed products.

Molecular docking and computational chemistry, including different chemometric tools, are crucial in Computer-Aided Drug Design (CADD). They have become a very important factor in the design and development of novel compounds with wide spectrum of biological activity, including anticancer, antibacterial and antifungal activities (3-5). The application of CADD approaches in early phases of development of biologically active compounds can make the search for leading compounds faster. Molecular docking is a CADD tool aimed to predict the possible interactions and predominant binding mode between a ligand and the virtual screening of a huge number a key protein. Its role in modern drug design is very significant in the virtual screening a huge number of molecules and their ranking (6). The molecular docking approach has been successfully applied in several studies to analyze antifungal activity of different compounds, such as triazole derivatives (7), 2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl dithiocarbamates (8), 1,3-thiazole derivatives (9), Ni²⁺, Co²⁺ and Cu²⁺ complexes of porphyrin core macromolecular ligand (10).

The present paper describes the application of the molecular docking procedure in the analysis of interactions between the newly synthesized 2-morpholinoquinoline derivatives and active site of glucosamine-6-phosphate synthase enzyme, which is considered to be the potential target for antifungal and antibacterial activity (11, 12). The main aim was to determine optimal positions of the analyzed ligands into the binding pocket of the target enzyme, to analyze the important interactions between the ligands and the enzyme, and to gain an overview on the molecular characteristics that contribute to their antifungal activity.

**MATERIAL AND METHODS**

The studied 2-morpholinoquinoline analogs and their antifungal activity against *Aspergillus fumigatus*

The analyzed 2-morpholinoquinoline analogs, which contain 1,2,4-oxadiazole, were synthesized by Karad et al (13). The results of antifungal activity of the studied analogs toward *Aspergillus fumigatus* have been taken from the literature (13). The antifungal activity was expressed as minimum inhibitory concentration (MIC) in mM units. The compounds of the highest interest were selected on the basis of the lowest MIC values. Ac-
According to this criterion, the analysis included the following compounds: (1) 2-(morpholino-4-yl)-3-{5-[4-(trifluoromethyl)phenyl]-1,2,4-oxadiazol-3-yl} quinoline, MIC = 0.146 mM (13), (2) 3-[5-(4-chlorophenyl)-1,2,4-oxadiazol-3-yl]-6-methyl-2-(morpholino-4-yl)quinolone, MIC = 0.246 mM (13) and (3) 2-(morpholino-4-yl)-3-{5-[3-(trifluoromethyl)phenyl]-1,2,4-oxadiazol-3-yl} quinolone, MIC = 0.234 mM (13). The analysis included griseofulvin (MIC = 0.283 mM) (13) as well, as a commercially available antifungal drug. The molecular structures of the studied compounds are presented in Figure 1. The compounds 1 and 3 are structural isomers which differ in the position of 4-trifluoromethyl group in the phenyl group. By comparing the compound 2 with the compounds 1 and 3, it can be seen that the compound 2 has an additional methyl group in the position 6 and chlorine in the position 4 in the phenyl group. The above compounds can be considered to have high antifungal potency toward \textit{Aspergillus fumigatus} fungus (13).

![Molecular structures of the analyzed 2-morpholinoquinoline analogs (1 - 3) and griseofulvin](image)

**Figure 1.** Molecular structures of the analyzed 2-morpholinoquinoline analogs (1 - 3) and griseofulvin

**Molecular docking analysis of 2-morpholinoquinoline analogs and griseofulvin**

The molecular docking modeling was done by using SwissDock software based on a web server EADock DSS (14). The CHARMM force field was used for the calculations (15), while the ranking was done on the basis of the most favorable binding energies. The X-ray structure of glucosamine-6-phosphate synthase enzyme was retrieved from the Protein Data Bank (PDB: \text{http://www.rcsb.org/}, PDB code 2VF5) (16). The analyzed ligands were energetically minimized by MM2 method, applying ChemBio3D software (17). The UCSF Chimera program was used for the visualization of the docking results (18). In order to validate the docking results, the molecular docking procedure was carried out in triplicate. The ligand-enzyme interactions were described by the following energies: Gibbs free energy ($\Delta G$), van der Waals energy ($\Delta G_{vdw}$), protein-solvent non-
polar energy ($\Delta G_{\text{protsolvnonpol}}$), ligand-solvent non-polar energy ($\Delta G_{\text{ligsolvnonpol}}$), $\Delta G_{\text{elec}}$ energy, ligand-solvent polar energy ($\Delta G_{\text{ligsolvpol}}$), protein-solvent polar energy ($\Delta G_{\text{protsolvpol}}$), compound-solvent non-polar energy ($\Delta G_{\text{compsolvnonpol}}$), surface-full energy, extra-full energy, protein-solvent polar energy ($\Delta G_{\text{compsolvpol}}$), intra-full energy, solvent-full energy, full-fitnes energy, inter-full energy, total energy, and simple-fitnes energy. According to the literature (19), in molecular docking studies the focus on $\Delta G$ is still considered “the safest bet”. Therefore, the results were interpreted by means of the $\Delta G$ values.

**Glucosamine-6-phosphate synthase (PDB: 2VF5)**

According to the X-ray analysis, in the binding pocket of glucosamine-6-phosphate synthase there are twelve amino acids (VAL399, GLN348, LYS603, GLY301, ALA602, ALA400, THR352, THR302, SER303, SER349, SER347 and CYS300) (11). This enzyme is known as L-glutamine : D-fructose-6-phosphate amidotransferase or under commonly used name glucosamine-6-phosphate synthase. It is a key enzyme in the catalysis of the reaction which involves ammonia transfer from L-glutamine to fructose-6-phosphate and following isomerization of the formed fructosamine-6-phosphate to glucosamine-6-phosphate (12). Earlier studies have shown that even a short-time inactivation of this enzyme in the cells of fungi is lethal due to induction of the lysis, agglutination, and certain morphological changes (12). The structure of glucosamine-6-phosphate synthase in the complex with glucosamine-6-phosphate, obtained by X-ray crystallography, is presented in Figure 2.

**Figure 2.** The structure of glucosamine-6-phosphate synthase in the complex with glucosamine-6-phosphate (PDB: 2VF5), which is marked by cyan color, retrieved from Protein Data Bank - PDB (http://www.rcsb.org)
RESULTS AND DISCUSSION

The molecular docking analysis of griseofulvin

The results of the docking of griseofulvin are given in Figure 3. It was determined that there are two main strong interactions between the ligand and the active site. The first one is a strong hydrogen bond between H-atom (HN) of THR302 and O-atom (O5) of the ligand. The length of this intra-molecular bond is 2.438 Å. The second one is a stronger H-bond than the previous one, with the length of 1.938 Å, between H-atom (HG1) of THR352 and O-atom (O6) of the ligand. The ΔG value of this interactions is -6.98 kcal/mol, followed by full fitness energy of -1643.10 kcal/mol and total energy of 25.26 kcal/mol. Also, there are certain lipophilic interactions present between the ligand and the active site; however, the hydrophilic interactions are predominant due to the chemical nature of the ligand, which can be considered more hydrophilic due to the presence of polar groups in its structure, such as metoxy, oxo and chlorine substituents.

![Figure 3. The docking of griseofulvin (cyan color) in the active site of glucosamine-6-phosphate synthase](image)

The molecular docking analysis of 2-(morpholin-4-yl)-3-{5-[4-(trifluoromethyl)phenyl]-1,2,4-oxadiazol-3-yl}quinoline (1)

The compound 1 was docked in the active site of glucosamine-6-phosphate synthase and it was determined that it forms several strong bonds with the active site (Figure 4). The presence of p-trifluoromethyl group enables several strong interactions between the ligand and active pocket of the enzyme. It can possibly form six strong H-bonds with the enzyme, concretely:

- three H-bonds between the F2-atom of the ligand and H-atom of THR352 (HG1) with the length of 3.120 Å, H-atom of SER349 (HN) with the length of 2.450 Å and H-atom of GLN348 (HN) with the length of 2.523 Å;
- two H-bonds between the F3-atom of the ligand and H-atom of THR352 (HG1) with the length of 2.543 Å, and H-atom of GLN348 (HN) with the length of 2.857 Å;
– one H-bond between the F1-atom of the ligand and H-atom of GLN348 (HN) with the length of 2.495 Å.

**Figure 4.** The docking of the compound 1 (cyan color) in the active site of glucosamine-6-phosphate synthase (a), and the zoomed interactions between the p-trifluoromethyl group and corresponding residues.

Besides the H-bonds formed between p-trifluoromethyl group and the binding site, there are two more significant interactions: (1) relatively weak H-bond formed between O-atom (O2) of the ligand and H-atom of SER401 (HN) with the length of 3.685 Å; (2) H-bond between N-atom (N3) of the ligand and H-atom (HN) of ALA602 of the length of 3.188 Å. The ΔG value of the interactions between the compound 1 and the binding site are described by a ΔG value of -7.54 kcal/mol, full fitness energy of -1609.35 kcal/mol, and total energy of 36.36 kcal/mol.

There are some weak lipophilic interactions between the non-polar rings of the ligand and certain residues of the binding pocket; however, the hydrophilic interactions are predominant. The p-trifluoromethyl group plays a very significant role in these interactions, enabling strong connections between the ligand and the residues of the binding pocket.

**The molecular docking analysis of 3-[5-(4-chlorophenyl)-1,2,4-oxadiazol-3-yl]-6-methyl-2-(morpholin-4-yl)quinoline (2)**

The compound 2 fits very well into the binding pocket of glucosamine-6-phosphate synthase; however it forms less inter-molecular bonds than the compound 1 (Figure 5). The compound 2, compared with the compound 1, has a chlorine substituent instead of the trifluoromethyl group in the position 4 of the 5-phenyl ring. Therefore, no significant interactions between the chlorine substituent and the neighboring residues have been determined. Nevertheless, there are some significant interactions between the ligand and the binding pocket which should be mentioned:

– strong H-bond between the O-atom (O2) of the ligand and H-atom (HN) of ALA602, with the length of 2.218 Å;

– relatively strong H-bond between the O-atom (O1) of the ligand and H-atom (HG1) of THR352, with the length of 3.317 Å.
The ΔG value of the present interactions between the compound 2 and the binding pocket is -7.32 kcal/mol. The full fitness energy is -1628.06 kcal/mol, and the total energy is 36.19 kcal/mol. As in the case of the compound 1, some weak lipophilic interactions between the non-polar rings of the ligand and certain residues of the binding pocket can be observed, but the hydrophilic interactions are predominant.

Figure 5. The docking of the compound 2 (cyan color) in the active site of glucosamine-6-phosphate synthase

The molecular docking analysis of 2-(morpholin-4-yl)-3-{5-[3-(trifluoromethyl)phenyl]-1,2,4-oxadiazol-3-yl}quinoline (3)

Despite the fact that the compound 1 and compound 3 are structural isomers, which differ only in the position of trifluoromethyl group in 5-phenyl ring, the difference between their antifungal activities against Aspergillus fumigatus is evident. This could be explained by the assumption that the p-trifluoromethyl group has much more possibility to interact with the residues than the m-trifluoromethyl group, as it is shown in Figure 4b.

Figure 6 indicates that the compound 3 forms four strong H-bonds between:
- F1-atom of the ligand and H-atom of THR352 (HG1) with the length of 2.805 Å;
- F2-atom of the ligand and H-atom of GLN348 (HG1) with the length of 2.402 Å;
- F3-atom of the ligand and H-atom of GLN348 (HE22) with the length of 2.773 Å;
- O2-atom of the ligand and H-atom of ALA602 (HN) with the length of 2.884 Å.

The docking of the compound 3 is described by a ΔG value of -7.33 kcal/mol. The full fitness energy is -1609.27 kcal/mol, and the total energy is 38.61 kcal/mol. The lipophilic interactions between the non-polar rings of the ligand and certain residues of the binding pocket are observable; however, in this case as well, the hydrophilic interactions are the most significant.
CONCLUSION

The presented results of the molecular docking analysis of the newly synthesized 2-morpholinoquinoline derivatives with antifungal potential against *Aspergillus fumigatus* are in agreement with the experimental findings. Namely, the analyzed compounds 1 - 3 expressed higher antifungal activity than griseofulvin, which is a commercially available antifungal therapeutic. This could be explained by the interactions which the newly synthesized derivatives realize with the binding pocket of the corresponding enzyme (glucosamine-6-phosphate synthase). Therefore, on the basis of the obtained results it can be concluded:

- The compound 1 expresses the highest antifungal potential against *Aspergillus fumigatus*, possibly due to the numerous strong H-bonds particularly achieved between the *p*-trifluoromethyl group and corresponding residues of the binding pocket;
- The compound 3 is a structural isomer of the compound 1; however, its antifungal activity is much lower than the antifungal activity of the compound 1. This could be explained by a higher possibility of the *p*-trifluoromethyl group in the compound 1 to form more H-bonds with the binding pocket than the *m*-trifluoromethyl group in the compound 3;
- The compound 2, due to the nature of the substituent in the position 4 in the 5-phenyl ring, has the lowest antifungal activity, compared to the compounds 1 and 3, but still higher than the antifungal activity of griseofulvin;
- The calculated Δ*G* energies are in agreement with the experimental results of antifungal activity. The docking of the compound 1, as the compound with the highest antifungal activity, showed the lowest Δ*G* value, and the docking of griseofulvin, as the compound with the lowest antifungal activity, was characterized by the highest Δ*G* energy. Therefore, the most active compound occupies the most energetically favorable position in the binding pocket.
The interactions between the ligands and the active pocket are predominantly hydrophilic;

The present results can be considered to be guidelines for further syntheses of 2-morpholinoquinoline derivatives structurally similar to the studied ones, as well as a contribution to the search of novel potent antifungal compounds which could find a wide spectrum of practical applications in medicine and agriculture.

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REFERENCES


АНАЛИЗА МОЛЕКУЛСКОГ ДОКИНГА НОВОСИНТЕТИСАНИХ 2-МОРФОЛИНОХИНОЛИНСКИХ ДЕРИВАТА СА АНТИФУНГАЛНИМ ПОТЕНЦИЈАЛОМ ПРЕМА Aspergillus fumigatus

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У овом раду представљена је анализа молекулског докинга новосинтетисаних 2-морфолинохинолинских аналога, који показују антифунгалну активност према Aspergillus fumigatus. Циљ ове анализе је да се установе потенцијалне интеракције између анализираних аналога и активног места ензима глукозамин-6-фосфат синтазе, као и да се утврде које молекулске карактеристике (присуство различитих супстишуена или изомерија) могу бити одговорне за значајну антифунгалну активност испитиваних јединења. Јединења са највишом антифунгалном активносту према патогеној плесни Aspergillus fumigatus докингована су у активно место ензима, а резултати су поређени са резултатима молекулског докинга грисеофулвина, антифунгалног терапеутика који се примењује за третман различитих типова дерматофитоза. Новооткривена антифунгална јединења су значајна како за медицину, тако и за пољопривреду. Превенција појаве микотоксина у прехрамбеним производима и храни за животиње један је од ургентних задатака. Стога, сваки допринос откривању механизма деловања новооткривених јединења и могућих штетних ефеката је драгоцен. Ова студија даје допринос анализи и селекцији новосинтетисаних антифунгалних јединења из групе 2-морфолинохинолина и пружа корак ка њиховој практичној примени у медицини и пољопривреди. Резултати представљени у овом раду откривају могући узрок јаче антифунгалне активности анализираних 2-морфолинохинолинских аналога према плесни Aspergillus fumigatus од антифунгалне активности грисеофулвина, као комерцијално доступног антифунгалног терапеутика.

Кључне речи: антифунгална активност, Aspergillus fumigatus, молекулски докинг, молекулско моделовање, плесни

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