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Heuristic analysis of structure, activity and selectivity relationships of isocoumarin derivatives as potential antifungal agents

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Abstract

A series of recently synthetized and biologically evaluated isocoumarin derivatives (n=16, including voriconazole as standard) were used for heuristic analysis of structure, activity and selectivity relationships. While research of isocoumarins found in the literature was mainly focused on antifungal properties of 3-alkyl/aryl compounds, this study used structures synthetized by a strategy merging antifungal properties of isocoumarins with some known fungal pharmacophores. Rationalization of activity and selectivity was performed using the results of testing against *C. albicans* (CA) and testing on a normal human lung fibroblast cell line (MRC5). Structures were created and optimized using the ChemDraw Ultra 8.0 and MOPAC software. Calculation of molecular descriptors and the heuristic method, using the Codessa software, were applied for the selection of the most significant descriptors for activity against CA, as well as selectivity against CA versus MRC5. Biological tests for determination of activity against CA used in studies include inhibition of hyphal growth, so the activity against resistant cells was also considered. The supposed mechanisms of action, including lacton opening and electrophilic attack on nucleophiles, as well as inhibition of lanosterol 14α-demethylase (CYP51), were in agreement with the results obtained. The results could serve as a basis for further optimization of isocoumarin derivatives with respect to better activity, selectivity and action against resistant species.

Key words: isocoumarins, heuristic analysis, *C. albicans*, CYP51, antifungals

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Introduction

Isocoumarins are a class of aromatic lactones which are of considerable interest due to their natural occurrence and a wide range of pharmacological activities, such as antifungal, anti-inflammatory, antimicrobial, phytotoxic, cytotoxic and other effects (1). Their applicability in the therapy of fungal infections, especially those caused by *C. albicans* (CA) and resistant fungi, is of particular interest, due to the fact that the fungal infection rate is increasing, which is especially true regarding immunocompromised individuals (2). The increased incidence of Candida infections can be attributed to a variety of factors. Over 100 species of Candida are known, whilst CA is the main representative. Since conventional fungal infection treatments are not satisfactory, it has become essential to develop new drugs and alternative therapies that would address the issues of overcoming toxicities / adverse effects and drug resistance associated with the currently available antifungals, which can limit the effectiveness of therapy (3).

In previous work, the focus was on creating new isocoumarin derivatives as potential antifungal compounds (4, 5). The isocoumarin skeleton was considered to be a privileged structure (6). In order to add to the previous studies, using sources from the literature and focusing on antifungal properties of 3-alkyl/aryl compounds of isocoumarins (7), synthetized compounds of this type were explored in greater detail, merging the antifungal potential of isocoumarins with some known antifungal pharmacophores (8, 9, 10, 11), which would provide combined action of those derivatives. Compounds were synthetized and tested against CA hyphal growth, which is responsible for penetration into human epithelial and endothelial cells during the initial phase of fungal infection and biofilm formation, which makes the fungus more resistant to standard treatments, therefore representing a suitable target for creating more effective antifungals. Azoles alone, despite being a first-line treatment for fungal infections, are also limited by drug resistance, so a new approach in designing isocoumarin derivatives as antifungals was supposed to overcome this obstacle. According to the results of testing, some azole substitute derivatives showed promising activity, while also being capable of inhibition of hyphal formation of CA. Testing of isocoumarin derivatives on normal cells (normal human lung fibroblast cell line, MCR5) was performed for estimation of selectivity on fungal cells, i.e., cytotoxicity of the compounds studied.

In this work, a heuristic analysis of structure, activity and selectivity relationships was performed using synthetized and biologically characterized isocoumarin derivatives, partially presented in our previous work (4, 5) and expanded for the purpose of further structure-activity studies. The interpretation of results was performed in light of closer insights into the supposed mechanisms of action against CA, as well as selectivity and toxicity with respect to MRC5, which could serve as a basis for further optimization of isocoumarin derivatives, with the aim of increasing activity and selectivity against fungal cells, as well as overcoming drug resistance.

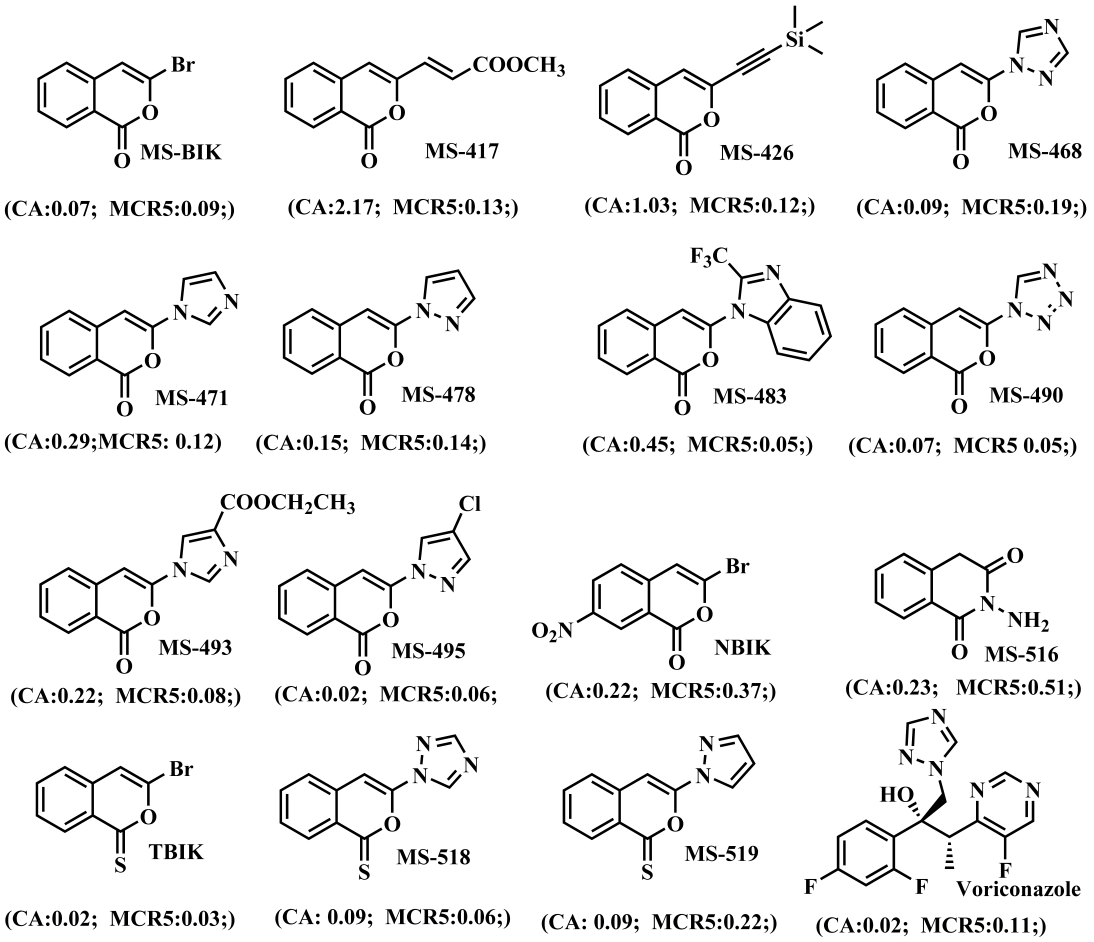
Experimental part

The structures of isocoumarin derivatives and voriconazole as standard (n=16) used for heuristic analysis (Figure 1) were drawn and cleaned to optimize bond lengths and angles using the ChemDraw Ultra 8.0 software (12). Geometrical optimization by the AM1 method (key words: vectors bonds pi polar precise enpart am1 nnmo) was performed using the MOPAC software (13). Calculation of descriptors (about 450 for most of the compounds) and heuristic analysis for selection of the most significant descriptors were performed using the Codessa software (14). The most significant descriptors, according to the coefficient of correlation, were selected among all descriptors and within the groups of descriptors: constitutional, topological, geometrical, electrostatic and quantum-chemical.

Results and discussion

A data set consisting of isocoumarin derivatives (Figure 1), including voriconazole as standard, synthetized by a strategy merging antifungal properties of isocoumarins with some known fungal pharmacophores and extended for the purpose of structure-activity analysis (4, 5), was used for heuristic analysis. Concerning the mechanism of action of isocoumarine derivatives, it was supposed to be based on two possible mechanisms. The first mechanism includes the opening of lacton structure, which acts as an electrophile attacking a nucleophile. Nucleophiles could be various biomolecules, having nucleophilic residues such as OH, NH_2, etc. (15). Concerning the structure-activity relationships of isocoumarins acting as electrophiles, the substituent at position 3 can influence electrophile activity by the electronic and steric effect. For example, when introducing bromine (Br), activity increases because of the negative inductive effect. The effect depends on the distance from lacton, so a derivative with a methyl bromine substituent does not show any activity against CA. The second possible mechanism of action of isocoumarins on CA is based on the presence of azoles, which represent a pharmacophore for antifungals (16). The main mechanism of azoles is the inhibition of CYP51, a key enzyme in fungal ergosterol biosynthesis. This in turn leads to depletion of ergosterol (a regulator of fungal cell membrane fluidity and asymetry) and accumulation of sterol precursors. As a result of ergosterol depletion, the integrity and function of fungal cell membrane are disrupted, eventually leading to cell lysis. In addition, ergosterol plays a hormone-like role in fungal cells, which stimulates growth, so the effect of azoles is the inhibition of fungal growth. Structure-activity relationships of various azoles are presented in the literature (17, 18, 19).

In order to get a deeper insight into the activities of newly synthetized isocoumarins, heuristic analysis was performed. The most significant descriptors among all descriptors were selected on the basis of correlation with activity against CA (classified as Ia). The most significant descriptors were also selected for each group of descriptors: constitutional, topological, geometrical, electronic and quantum-chemical (classified as Ib). This approach was supposed to provide main and branching information in the network of factors influencing the activity of compounds against CA.



MS-BIK 3-Bromo-1*H*-isochromen-1-one

MS-417 (*2E*)-methyl 3-(1-oxo-1*H*-isochromen-3-yl) acrylate

MS-426 3-[2-(trimethylsilyl)ethynyl]-1*H*-isochromen-1-one

MS-468 3-(1*H*-1,2,4-triazol-1-yl)-1*H*-isochromen-1-one

MS-471 3-(1*H*-imidazol-1-yl)-1*H*-isochromen-1-one

MS-478 3-(1*H*-pyrazol-1-yl)-1*H*-isochromen-1-one

MS-483 3-(2-(trifluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-1*H*-isochromen-1-one

MS-490 3-(1*H*-tetrazol-1-yl)-1*H*-isochromen-1-one

MS-493 Ethyl 1-(1-oxo-1*H*-isochromen-3-yl)-1*H*-imidazole-4-carboxylate

MS-495 3-(4-chloro-1*H*-pyrazol-1-yl)-1*H*-isochromen-1-one

NBIK 7-Nitro-3-(1*H*-1,2,4-triazol-1-yl)-1*H*-isochromen-1-one

MS-516 2-Amino-4*H*-isoquinoline-1,3-dione

TBIK 3-Bromo-1*H*-isochromene-1-thione

MS-518 3-(1*H*-1,2,4-triazol-1-yl)-1*H*-isochromene-1-thione

MS-519 3-(1*H*-pyrazol-1-yl)-1*H*-isochromene-1-thione

Figure 1. Isocoumarin derivatives used for QSAR studies, tested against CA and on MRC5. Activities are expressed as MIC [mM] for CA and IC50 [mM] for MCR5 (in brackets) (4, 5).

Slika 1. Derivati izokumarina korišćeni za *QSAR* studije, testirani protiv *CA* i na *MCR5*. Aktivnosti su izražene kao MIC [mM] za *CA* i IC5*0* [mM] za *MCR5* (u zagradi) (4, 5).

Molecules with Br at position 3 show activity as well as molecules with azoles at position 3, which could be proof that molecules act by two mechanisms. Concerning the first mechanism of action, electronic and steric properties of substituents at position 3 influence lacton opening, and therefore their activity against CA. A few selected descriptors belonging to Ia and Ib could be the proof that molecules act by the proposed mechanism. *Polarity parameter/square distance* (r=0.2847, Ib) could be a measure of the ability of a molecule for polarization on partially positive carbon ($C^{\delta+}$) and partially negative oxygen ($O^{\delta-}$) as part of lactone. The more polar a carbon group of lacton is, the higher the possibility of opening. The selected descriptors *WNSA-3** (r=0.4771, Ib), a quantum-chemical descriptor providing numerical vector representation of geometrical features of a molecule, but at the same time describing the electronic features that affect intermolecular interactions, as well as *WPSA-2** (r=0.3759, Ib), are important descriptors reflecting steric and electronic properties of the molecules in the network of compound occurrences. The significance of *WPSA-2* could be the proof that the surface of an electrophile created after lacton opening is important for action.

Concerning the second mechanism of action, azole drugs bind to the CYP51 active site through coordination of a heme iron by N-heterocycle nitrogen. The fitting of azoles with enzyme at first instance depends on the size and shape of molecules. Descriptors reflecting the size of molecules are *Molecular surface area* (r=0.223, Ib) and *Molecular volume* (r=0.1841, Ib). Constitutional descriptors such as the *Number of atoms* (r=0.2153, Ia) also indicate that the size of a molecule, depending on the size of variable substituents, is important for azole-enzyme interaction. For fitting within the precise geometry of an active site, geometry of molecules is also essential. Geometrical descriptors reflecting the orientation of molecules are also selected as important: *ZX shadow* (r=0.2550, Ia), as well as *XY shadow* (r=0.2321, Ia). The active site of CYP51 contains a heme cofactor and binding of ligands requires accommodation, depending on the adjustment to the CA shape (Ref 20, Figure 8). During binding, the heme sixth position of octahedral coordinate geometry of iron (Fe, *ferrum*) can be occupied by nitrogen (N) (for example of imidazole ring or triazole ring of azole drugs). The binding of azole occurs by a stepwise mechanism (21). First, a water molecule from resting state of P450 is released from the sixth binding site of the heme to create a pentacoordinated active site, followed by coordination of azole N to the heme iron Fe. This process leads to breaking hydrogen bonds between the resting state water molecule and approaching inhibitor molecule. When the azole molecule approaches the heme closely, the water molecule is released from the heme, and a pentacoordinated heme group remains, with nearby azole and water. Azole then replaces water in the sixth ligand position of the heme, thereby blocking dioxygen binding and rendering the heme centre inactive. Perturbations within the active site (polarized environment) happen during the replacement of water with azole. Azoles bind the heme with significantly stronger binding energies than a water molecule, so inhibitors block the catalytic site of the enzyme. The binding of azoles, however, depends on their capability for polarization, relative to low polarized water. It is shown that *GAMMA* and *BETTA polarizability* are among the most significant

descriptors (r=0.4770 and r=0.4445, respectively, Ia). Those descriptors reflect the capability of their electronic system to be distorted by the external field. Tightness of the interaction between N and Fe depends on conformation flexibility, due to limiting plasticity of the binding pocket. Various azoles adjust their position in the pocket, changing its shape and size to better fit the drug molecule. Thus, tight binding to CYP51 of azole antifungals seems to depend on the ability of the drug to adopt a conformation that accommodates the hydrophobic pocket in the CYP51 active site and places the drug's main backbone along an imaginary axis that runs down the middle of the CYP51 substrate access channel. Therefore, it is not surprising that the most significant descriptors are the *Kier flexibility index* (r=0.4812, Ia) and *Kier shape index* (r=0.4308, Ia). *Kier flexibility index*, as a measure of molecular flexibility, is derived from the *Kier shape indices*, encoding information about molecular size, branching, cycles and heteroatom content. The more flexible a molecule is, the more influence it will have on the orientation of the molecule towards the enzyme. Since the active site of the enzyme consists of a substrate channel (which connects the heme group and protein surface) and a binding site, the right orientation of the molecule could provide full access to binding. *Moment of Inertia C* (r=0.1653, Ib) and *Moment of Inertia B* (0.1558, Ib), which are the measure of rotational inertia of a rigid body, are selected among the most significant descriptors as well; this indicates that rotation around a rigid body determines the probability of forming an active conformation. Concerning the formation of a coordinative bond, it involves a ligand with N possessing a free electronic pair shared with Fe of the heme. The electronic pair is attracted by both nuclei. During the formation of a coordinative bond, orbitals overlap (sp^2 orbital of azoles and d orbital of Fe), so the strength of binding depends on the distance between the ligand and metal. The ability to form a coordinative bond could be described by *Bonding information content* (r=0.3595, Ib) and *Complementary information content* (r=0.1848, Ia). *Complementary information content* describes the number of atomic layers in the coordination sphere around a given atom that are accounted for, whilst *Bonding information content* describes the number of atomic layers in the coordination sphere around a given atom that are accounted for related to the number of edges in the molecular graph. *Relative number of rings* (r=0.3179, Ib), *Relative number of benzene rings* (r=0.2047, Ib) and *Relative number of aromatic bonds* (r=0.2003, Ib) also appeared to be important, which might indicate that the activity of compounds is influenced by the fit of the aromatic part to the hydrophobic groove of CYP51. Actually, the lipophilic part of a molecule mimics non-steroidal natural subtrate lanosterol, part of the mechanism that is described above. Moreover, aromatic rings could increase lipophilicity, which is also important for antifungal activity. Since azoles are introduced at position 3, they also influence electrophilic activity of the isocumarin ring by the electronic and steric effect. However, the direct proof that derivatives act by inhibiting the enzyme is that compounds with sulfur (S) instead of O, which are more electronically stable and therefore cannot produce an electrophile, also show significant activity.

Concerning the selectivity of isocoumarins derivatives, which should be considered for toxicity estimation, there are various mechanisms by which those compounds could interact with other targets, besides those located in fungus cells. First, CYP51 is present not only in fungi, but in many other species, including humans (22), in which this enzyme is involved in the biosynthesis of cholesterol, the major sterol of mammalian membranes. Thus, one of the requirements for antifungal azoles is to inhibit fungal CYP51, but avoid or minimize the inhibition of the human ortholog that metabolizes the same substrate lanosterol. Second, at therapeutic concentrations, antifungal azoles should also not inhibit other P450 isoforms present in humans that play important roles in the metabolism of endogenous and exogenous compounds. Currently, however, this is not the case, and this is one of the limitations of antifungal azoles. Even the second and third generation antifungal azoles inhibit some of the host P450s, e.g., human CYP3A4, CYP2C9, and CYP2C19 (23), which could also be reason for side effects on human cells. Third, due to the reactivity of lacton as an electrophile after opening, isocoumarins could act on various biomolecules (24), though their selectivity is based on cumulation in fungal cells, perhaps depending on the specific transport.

Cytotoxicity of isocoumarin derivatives synthesized was tested on normal MRC5 cells. The selectivity of compounds is not remarkable, but insights into the mechanism of cytotoxic activity would be helpful for optimization, in order to increase their selectivity. It could be concluded from the results that all the abovementioned mechanisms could play a role in cytotoxicity of the compounds on normal cells. The heuristic method was applied for selecting the most significant among all descriptors (group IIa), and the most significant descriptors within the group of descriptors (IIb) for activity on MRC5.

Concerning the selectivity between species, there are indications that molecules interact with Fe of the heme in CYP51 of normal cells. According to topological descriptors *Average Bonding Information Content* (r=0.209, IIa) and *Average Complementarity Information Content*, (r=0.1650, IIa), molecules act by creating coordinative bonds with Fe of the heme. The importance of bioactive conformation, due to the configuration of the binding pocket, could be demonstrated by the descriptors reflecting molecular surface size (*Molecular Surface Area* (r=0.1156, IIa)), and molecular volume particle divided in XYZ box (*Molecular volume/XYZ Box* (r=0.2241, IIb)).

However, the low selectivity of isocoumarins could be due to the activity on various isoforms of P450, besides CYP51. The active place of P450 isoforms is highly conserved, whilst variations in primary sequence might have a significant influence on configuration and type of interactions with the binding pocket. It was shown that the activity on MRC5 cells depends on different descriptors from those for activity against CA. Even if the mechanism for binding to the heme is included, different effects are associated with various CYP51 isoforms. For example, *HOMO energy** (r=0.5485, IIa), *min (#HA,#HD)** (r=0.5293, IIa) are the most significant descriptors for activity on MCR5, which indicates different interactions, including optical factors, electricity conduction, and hydrogen bonding. The selected descriptor *XY Shadow/XY rectangle* (r=0.5245, IIb) reflects geometrical requirements for binding.

Broad activities of isocoumarins on MRC5, due to the opening of lacton and forming an electrophile that interacts with various biomolecules, could be reflected in the following descriptors: *Min coulombic interaction for a C-O bo*nd, (r=0.4832, IIa), *Min partial charge for an atom* (r=0.4102, IIb), *RNCS**, (r=0.3442, IIb), *FNSA-3**(r=0.5221, IIa). Coulombic interaction refers to the primary force that determines the behavior of colliding atoms or molecules, acting between constituent electrons and nuclei. It is a measure of Coulomb potential and plays a crucial role in collision dynamics. It could be an important piece of information for further optimization of selectivity of the compounds investigated, in order to lower their primary invasion. However, the results on MCR5 are relative, since in a network with a rising level of CA the compounds would perhaps primarily take orientation on that target. The question of dose is a matter of harming CA taking into account its rising level and role, while keeping normal cells unharmed. *FNSA-3*, as another molecular orbital related descriptor, leads to the conclusion that negative areas related to the total molecular area of molecules, depending on the number of bonding electrons, are important for activity. *Min atomic state energy for a O atom* (r=0.5179, IIb), *Min resonance energy for a C-O bond* (r=0.5097, IIb) and *Min e-n attraction for a O atom* (r=0.4921, IIb) suggest dynamic electron transfer happening in these actions.

Heuristic analysis of isocomarines was also performed in order to investigate what molecular properties are important for selectivity of the compounds against CA versus MRC5. The most significant of all descriptors (IIIa) and the most significant descriptors within the groups (IIIb) were selected relative to the ratio of activity CA/MRC5. *Kier flexibility index* (r=0.6720 IIIa) index and *Kier shape index* (r=0.6617, IIIa) were selected as the most important, determining the orientation and shape of active conformation. Descriptors *1X GAMMA polarizability* (r=0.5089, IIIa), which is important for the replacement of water, as well as *Complementarity information content* (r=0.3840, IIIa), describing the ability to form a coordinative bond between N and Fe, are also selected among the most significant descriptors for selectivity against CA. Electronic factors are alo important for selectivity, coded in the following descriptors: *Topographic electronic index (all bonds)* (r=0.3849, IIIb), *PPSA-1** (r=0.3819, IIIb) and *WPSA-2** (r=0.6074, IIIb).

Descriptor *Tot molecular 2-center exchange energy* (r=0.3873, IIIb) could be a measure of the strength of the exchange interaction between neighboring spins, which tries to maintain their magnetic moments parallel to one another, whilst *Tot molecular 2-center resonance energy* (r=0.3546, IIIb) reflects the electronic transfer. *Molecular volume* (r=0.3755, IIIb*)* and *Molecular volume / XYZ Box* (r=0.16944, IIIb) are both

* Full name of descriptors in order of appearance in the text: WNSA-3 (Weighted Polar Negative Surface Area [Semi-MO PC]); WPSA-2 (Weighted Polar Positive Surface Area [Zefirov's PC]); HOMO energy (Highest Occupied Molecular Orbital energy); min (#HA, #HD) (Minimum value of the count of Hydrogen-Acceptor sites and the count of Hydrogen-Donor sites); RNCS (Relative Negative Charged Surface Area [Zefirov's PC]); FNSA-3 (Fractional Polar Negative Surface Area/Total Molecular Surface Area [Semi-MO PC]); PPSA-1 (Partial positive surface area [Zefirov's PC]): WPSA-2 (Weighted Partial Positive Surface Area (PPSA2*TMSA/1000 [Zefirov's PC]), TMSA-Total Molecular Surface Area;

important. Descriptors *Moment of inertia C* (r=0.2366, IIIb) as a measure of rotation energy and *ZX Shadow* (r=0.4462, IIIb) indicate that more space rearrangements happen within the process of selective action.

Only a few compounds showed activity against resistant species, such as *C. krusei* and *C. parapsilosis*, which indicates that a significant improvement has been achieved concerning the activity against resistant cells. However, the number of isocoumarin compounds showing activity against resistant species is not sufficient for heuristic analysis, but could serve as a base for further optimization of more compounds that would provide the rationale for activity against resistant cells.

As for activity, selectivity and toxicity studies, the approach of using the heuristic method of ranking descriptors regarding their significance among classes and within classes seemed to cross subtle lines between the main and supportive determinants for dependents regarded. Overall, the results indicate that prevailing factors for the activity of compounds investigated can possibly be attributed to the ability of CA to change shape and construct various types of membranes through a stepwise mechanism (25).

Besides the interpretation presented in study, some further factors should also be regarded, taking into account the optimization of possible leads, and its application on another interaction of the human organism and changing environment. In recent studies (26), a remarkable review of advances in the therapeutic potential of isocoumarins derived from fungi was presented, merging its application for various biological and pharmacological activities. The issue of biological and pharmacological activities can further be considered, since isocoumarins originating from various fungi depend on the communication of fungi and other factors. Although therapy of CA can be justified for some period of time, its higher level might indicate a certain role in functionalization or re-functionalization of the contaminated organism, whilst the output depends on numerous factors. Rising antifungal resistance and tolerance of CA (27) can be an alert for consideration of dosage and duration of trials, taking into account the balance between the inhibition of CA and the roles CA plays in the organism, depending on various human organism states. The level of CA resistance (due to biofilming and mutations) and CYP isoforms might indicate a network of co-activities for a certain purpose. Therefore, the approach of a double mechanism inhibiting ergosterole synthesis, given in our studies, might seem promising in remodeling the rising resistance trends within the human organism, and possibly providing a more sound and simplified level of patients states for further consideration of causes and consequences of CA contamination and related factors.

The results of heuristic analysis, however, supported the supposed double mechanism of isocoumarin derivatives studied in this work. Concerning selectivity, which depends on the ratio of their activity on the fungal CYP51 versus human CYP51 enzyme, as well as the ratio of activity on CYP51 versus other isoforms of P450 enzymes, strength of intermediate electrophiles and other factors, heuristic analysis could aid in finding solutions for further optimization of compounds given in the study. Concerning resistance, its appearance should be considered within a broad context of the interplay

between CA roles, reasons for possible adaptation and mutations, with necessary treatments including subtle distinctions supported by mechanistic studies of whole processes, and further consideration of the causes.

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Declaration of Competing Interests

The chemical structures of isocoumarin derivatives used in this study were created by the team of prof. Vladimir Savić (Department of Organic Chemistry, University of Belgrade – Faculty of Pharmacy), who retain intellectual property rights.

Author contribution

Author Milena Simić has contributed to conceptualization, data curation and investigation. Author Slavica Erić has contributed to conceptualization, formal analysis, investigation and original draft writing.

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Heuristička analiza odnosa strukture, aktivnosti i selektivnosti derivata izokumarina kao potencijalnih antifungalnih agenasa

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Kratak sadržaj

Serija skorašnje sintetisanih i biološki testiranih derivata izokumarina (br. 16, uključujući vorikonazol kao standard) korišćena je za heurističku analizu odnosa strukture, aktivnosti i selektivnosti. Istraživanja izokumarina pronađena u literaturi su uglavnom fokusirana na antifungalne osobine 3-alkil/aril derivata, dok su u ovim studijama korišćene strukture sintetisane strategijom kombinovanja antifungalnih osobina izokumarina sa nekim poznatim fungalnim farmakoforama. Racionalizacija aktivnosti i selektivnosti vršena je korišćenjem rezultata testiranja protiv *C. albicans (CA)* i na normalne humane ćelije plućnog fibroblasta (*MRC5*). Strukture su kreirane i optimizovane korišćenjem *ChemDraw Ultra 8.0* i *MOPAC* softvera. Izračunavanje molekulskih deskriptora i heuristička metoda, korišćenjem *Codessa* softvera, primenjeni su za selekciju najznačajnijih deskriptora za aktivnost protiv *CA*, kao i selektivnost protiv *CA* u odnosu na *MCR5*. Biološki testovi za određivanje aktivnosti protiv *CA* korišćeni u studijama uključuju inhibiciju hifalnog rasta, te je razmatrana i aktivnosti protiv rezistentnih ćelija. Pretpostavljeni mehanizmi aktivnosti, uključujući otvaranje laktona i elektrofilni napad na nukleofile, kao i inhibiciju lanosterol 14α-demetilaze (*CYP51*), u saglasnosti su sa dobijenim rezultatima. Rezultati mogu da posluže kao osnova za buduću optimizaciju derivata izokumarina u cilju postizanja bolje aktivnosti, selektivnosti i dejstva protiv rezistentnih vrsta.

Ključne reči: izokumarini, heuristička analiza, *C. albicans*, CYP51, antifungali