

Structure-Activity Relationship of the Antifungal 1-Aryl-2-(azol-1-yl)ethane Derivatives

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The relationship between the chemical structure of 54 1-aryl-2-(azol-1-yl)ethane derivatives and their antifungal activities, tested on 30 fungal species, have been examined. Among the 1-keto derivatives the compounds with a longer alkyl chain at the carbon atom adjacent to the azole ring as well as the chloro substituent on the benzene ring showed the highest antifungal activity, meanwhile, a shorter chain at the same carbon atom led to highest activity among the 1-chloro derivatives. To find the physico-chemical properties influencing the antifungal activity two series of molecular descriptors have been calculated applying the Dragon and the Sybyl VolSurf computer programs. The 3D structures of the compounds to these calculations were generated with the HYPERCHEM and the SYBYL molecular modeling programs. From the equation, obtained with stepwise regression using the antifungal activity data as dependent and the descriptors as independent variables, it is obvious that the factors describing the chemical structure itself (Dragon descriptors) have greater influence on the antifungal activity than the factors responsible for the passive penetration (VolSurf descriptors). The principal component analysis revealed that the 1-chloro derivatives, which are equipotent or more potent than the 10 reference azole fungicides, have at least two action modes.

Keywords: azole fungicides, structure-activity relationship, Volsurf descriptors, Dragon descriptors, molecular modeling, Sybyl, HyperChem.

Since the discovery (Vanbreuseghen et al., 1967) of the antifungal properties of substituted azole derivatives in the late sixties of the last century, many imidazole, 1,2,4-triazole, pyridine and pyrimidine derivatives have become active ingredients of agricultural fungicides or human medicines. The primary mode of action of this type of compounds is the inhibition of cytochrome P450 14 α -sterol demethylases (CYP51) catalyzing the oxidative removal of the 14 α -methyl group of lanosterol in the ergosterol biosynthesis of fungi, yeast and other microorganisms, respectively (Buchenauer, 1977; Gadher et al., 1983; Van den Bossche, 1985). The development of great number of commercial DMI (demethylase inhibitor) fungicides was helped by the studies of the relationship between their chemical structures and antifungal activities (Brown et al., 1984; Marchington, 1984; Fujimoto et al.,

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1988; Nakayama, 1992). The fact that the crystal structure of 14 α -sterol demethylase has recently become known (Podust et al., 2001) can help to design new azole fungicides.

There exists a structural unit, i.e., a two-carbon bridge, connecting the azole ring to an aromatic ring, which are common to most of the azole fungicides. In the structure activity relationship studies the effect of alkyl substituents to the antifungal activity on the carbon atom adjacent to the azole ring has not examined thoroughly. We herein describe the relationship between the chemical structure of 1-aryl-2-(azol-1-yl)ethan-1-one, -1-ol and -1-chloro derivatives bearing alkyl substituents on the carbon atom adjacent to the azole ring and the average antifungal activity (AAA) measured on thirty fungal species. The 1-aryl-2-(azol-1-yl)ethan-1-one derivatives have been also shown to inhibit different P450 monooxygenases from insects too (Bélai et al., 1988; Darvas et al., 1992). Our new derivatives are expected to possess new action mode(s), too. Compounds having multisite action modes can more easily take the fight up against the acquired resistance than those having only single mode of action.

Materials and Methods

Chemicals

The azole derivatives **1–54** were synthesized in our laboratories. Their structure together with their AAA data are collected in *Table 1*. The synthesis of the keto ($X = C=O$) and hydroxy ($X = CH-OH$) derivatives were carried out by the methods described in our earlier work (Bélai et al., 1995). The chlorine derivatives ($X = CH-Cl$) were obtained by the reaction of the hydroxy derivatives with thionyl chloride. The structure of the compounds were identified with IR and 1H -NMR spectroscopy as well as elemental analysis. Their purity was checked by thin layer chromatography in two developing systems. The details of the synthesis, as well as the spectroscopic data of the azole derivatives will be published elsewhere.

The reference fungicides listed in *Table 1* were isolated from appropriate commercial preparations obtained from the market and identified by their melting point.

Antifungal activity

The fungi tested and their taxonomic positions according to Hawksworth et al. (1995) are listed in *Table 2*, and all of them were taken from the collection of Plant Protection Institute of the Hungarian Academy of Sciences (Budapest, Hungary). The test species represent various taxonomic groups as well as modes of habitation (Parberry, 1996). Fungi were maintained on Potato-Dextrose Agar (PDA) slants, whilst Oomycetes on Green Pea Agar (GPA) slants. For antifungal activity tests, the PDA was inoculated with fungus (10^5 propagules per 20 ml of medium), and a layer (depth, 5 mm) was dispensed into Petri dishes (diameter, 90 mm). Filter paper discs (diameter, 5 mm) impregnated with 10^{-6} M of test compounds were placed centrally on the agar plate (one disk per dish), and growth-inhibition zones were measured after incubation at 22 ± 1 °C for 48 h.

Table 1

The structure and average antifungal activity of the test compounds
and the reference commercial fungicides

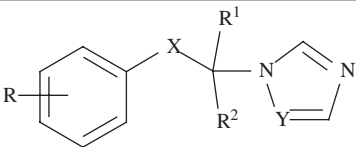
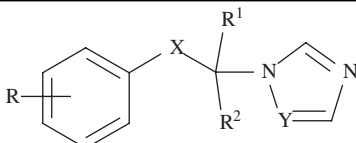
No.						Average Antifungal Activity ^a (%)	ESA ^b
	Structures						
	R	R ¹	R ²	X	Y		
1	H	Me	Me	C=O	CH	1.9	6.8
2	H	Me	Me	CH-OH	CH	1.5	5.6
3	H	Me	Me	CH-Cl	CH	15.9	20.1
4	H	Me	Me	CH-OAC	CH	3.5	6.4
5	H	Me	Me	C=O	N	0.9	3.9
6	H	Me	Me	CH-OH	N	2.1	11.0
7	H	Me	Me	CH-Cl	N	4.6	18.2
8	H	Me	H	C=O	CH	1.0	2.9
9	H	Me	H	CH-OH	CH	0.3	1.4
10	H	Me	H	CH-Cl	CH	18.1	20.6
11	H	Me	H	C=O	N	1.3	6.1
12	H	Me	H	CH-OH	N	1.8	9.5
13	H	Me	H	CH-Cl	N	1.3	3.3
14	H	H	H	C=O	CH	0.7	2.8
15	H	H	H	CH-OH	CH	0.9	4.6
16	H	H	H	C=O	N	0.5	2.2
17	H	H	H	CH-OH	N	0.7	3.4
18	H	Et	H	C=O	CH	3.2	6.0
19	H	<i>i</i> -Bu	H	C=O	CH	13.2	19.6
20	<i>p</i> -MeO	Me	Me	C=O	CH	1.0	2.0
21	<i>p</i> -MeO	Me	Me	CH-OH	CH	0.1	0.1
22	<i>m</i> -MeO	Me	Me	C=O	CH	0.2	0.1
23	<i>m</i> -MeO	Me	Me	CH-OH	CH	0.1	0.5
24	<i>p</i> -MeO	Et	H	C=O	CH	6.1	9.5
25	<i>p</i> -Cl	Me	Me	C=O	CH	6.7	10.4
26	<i>p</i> -Cl	Me	H	C=O	CH	1.8	2.4
27	<i>p</i> -Cl	Me	H	CH-Cl	CH	29.8	26.4
28	<i>p</i> -Cl	Me	H	C=O	N	0.7	2.1
29	<i>p</i> -Cl	Me	H	CH-OH	N	0.4	1.2
30	<i>p</i> -Cl	Et	H	C=O	CH	13.9	19.7
31	<i>p</i> -Cl	Et	H	CH-Cl	CH	28.6	25.1
32	<i>p</i> -Cl	<i>n</i> -Bu	H	C=O	CH	22.8	24.4
33	<i>p</i> -Cl	<i>n</i> -Bu	H	CH-OH	CH	9.6	17.9
34	<i>p</i> -Cl	<i>n</i> -Bu	H	CH-Cl	CH	15.7	19.6
35	<i>p</i> -Cl	H	H	C=O	CH	1.5	5.3
36	<i>p</i> -Cl	H	H	CH-OH	CH	4.2	16.4
37	<i>p</i> -Cl	H	H	CH-Cl	CH	19.8	23.3
38	<i>p</i> -Cl	H	H	C=O	N	0.3	0.7
39	<i>p</i> -Cl	H	H	CH-Cl	N	12.4	20.6

Table 1 (cont.)

No.						Average Antifungal Activity ^a (%)	ESA ^b
	Structures						
	R	R ¹	R ²	X	Y		
40	2,4-di-Cl	Me	H	C=O	CH	13.5	19.2
41	2,4-di-Cl	Me	H	CH-OH	CH	15.1	20.8
42	2,4-di-Cl	Me	H	CH-Cl	CH	35.5	25.6
43	2,4-di-Cl	H	H	CH-Cl	N	18.8	25.6
44	<i>p</i> -NO ₂	Me	Me	C=O	CH	3.0	9.3
45	<i>p</i> -NO ₂	Me	Me	CH-OH	CH	1.1	0.1
46	<i>t</i> -Bu	Me	Me	C=O	CH	5.0	11.8
47	<i>t</i> -Bu	Me	Me	CH-OH	CH	1.7	1.1
48	<i>p</i> -PhCH ₂ O	Me	Me	C=O	CH	7.4	19.7
49	<i>p</i> -PhCH ₂ O	Me	Me	CH-OH	CH	6.3	14.4
50	<i>p</i> -PhCH ₂ O	Me	Me	C=O	N	1.1	3.4
51	<i>p</i> -PhCH ₂ O	Me	H	C=O	CH	9.9	18.3
52	<i>p</i> -PhCH ₂ O	Me	H	CH-OH	CH	6.1	14.2
53	<i>p</i> -PhCH ₂ O	Me	H	C=O	N	3.3	8.3
54	<i>p</i> -PhCH ₂ O	Me	H	CH-OH	N	2.3	7.3
Reference Compounds							
Bitertanol						9.5	15.6
Imazalil						32.3	27.6
Clotrimazole						12.2	17.7
Myclobutanyl						21.1	25.2
Paclobutrazole						20.1	26.4
Prochloraz						32.6	28.8
Propiconazole						27.8	27.1
Triadimefon						18.2	27.3
<i>erythro</i> -Triadimenol						18.3	27.3
<i>threo</i> -Triadimenol						6.6	13.0

^a The basic data matrix on activity of compounds (N=58×30) is not shown. The variation coefficients of measurements did not exceed 4% in majority of cases.

^b The extent of spectrum of activity (ESA) was characterized with deviation of average activity measured on 30 test species. The selectivity of compounds is proportional to the size of number.

For anti-oomycete tests, the GDA medium was inoculated with *Pythium irregulare* or *Phytophthora cryptogea* mycelium suspension (1 ml/dish) and a layer of the medium (depth, 5 mm) was dispensed into Petri dishes (diameter, 90 mm). Filter paper disks (diameter, 5 mm) were impregnated with 10⁻⁶ M of test compounds and placed centrally on the agar plate (one disk per dish). The growth inhibition zones were measured after incubation at 18 ± 1 °C for 72 h.

All experiments were carried out at least three times. The antifungal activity of a single compound was calculated as a percentage of area of inhibition zone related to the total surface of Petri dish. The average antifungal activity (AAA), which is the mean of

Table 2

The fungal species and their origin

No. Species	Origin
Oomycota (Eukaryota, Protoctista)	
1. <i>Pythium irregulare</i> Buism	pea
2. <i>Phytophthora cryptogea</i> Pethyb et Laff.	azalea
Zygomycota (Eukaryota, Fungi)	
3. <i>Mucor racemosus</i> L.	air
4. <i>Rhizopus nigricans</i>	paprika
Basidiomycota (Eukaryota, Fungi)	
5. <i>Schroeteria decaisneana</i> (Boudier) de Toni	<i>Viola tricolor</i>
6. <i>Ustilago maydis</i> (De Candolle) Corda	maize
Ascomycota (Eukaryota, Fungi)	
7. <i>Saccharomyces cerevisiae</i> Hansen	yeast
8. <i>Penicillium oxalicum</i> Currie et Thom	orange
9. <i>Thielaviopsis basicola</i> (Berk. et Broome) Ferraris	tobacco
10. <i>Fusarium graminearum</i> Schwabe	maize
11. <i>Fusarium oxysporum</i> Schlechtendahl	tomato
12. <i>Fusarium solani</i> Reinke et Berthold	potato
13. <i>Colletotrichum lindemutianum</i> Saccardo et Magnus	bean
14. <i>Colletotrichum atramentarium</i> (Berk. et Br.) Tankenhanse	tomato
15. <i>Colletotrichum dematium</i> (Pers.) Grove	soya
16. <i>Colletotrichum gloeosporioides</i> Penzig	horseweed
17. <i>Cladosporium cucumerinum</i> Ellis et Arthur	cucumber
18. <i>Botrytis allii</i> Munn	onion
19. <i>Botrytis cinerea</i> Pers., Benomil resistant	paprika
20. <i>Botrytis cinerea</i> Pers., Benomil sensitive	grape
21. <i>Aspergillus niger</i> van Thieg	onion
22. <i>Ascochyta pisi</i> Libert	pea
23. <i>Trichotecium roseum</i> Link ex Fries	apple
24. <i>Didymella applanata</i> (Niesl) Saccardo	raspberry
25. <i>Venturia inaequalis</i> Cke.	apple
26. <i>Piricularia orizae</i> Briosi et Cavara	rice
27. <i>Alternaria solani</i> (Ellis & Martin) Jones et Grout	paprika
28. <i>Septoria lycopersici</i> Spegazzini	tomato
29. <i>Verticillium albo-atrum</i> Reinke et Berthold	tomato
30. <i>Fulvia fulva</i> Cooke (Cif.)	tomato

activities measured on 30 test species, was used for QSAR studies. Details other than here are outlined elsewhere (Oros et al., 2002).

Molecular modeling and descriptors

The molecular descriptors were calculated by means of the software package DRAGON 5.3 program available in the Web for evaluation (Todeschini et al., 2005),

which provides 1481 molecular descriptors (Todeschini and Consonni, 2000). The three-dimensional structures of the compounds to these calculations were modeled with the HYPERCHEM program package (HyperChem, 1997) and their geometry was minimized with molecular mechanics calculations using the MM+ method and a gradient of $0.01 \text{ kcal mol}^{-1} \text{ \AA}^{-1}$ as termination criterion. After exclusion of those with zero or constant values the remainings were correlated with the AAA, and those whose correlation coefficient (r) values are equal or greater than 0.5 have been chosen for stepwise regression examination.

The pharmacokinetically-relevant molecular descriptors were calculated with VolSurf, selecting H_2O (water), DRY (hydrophobic), O (carbonyl oxygen), BOTH (amphipathic), and O: (carboxyl oxygen) as probes. Grid spacing was $0.5 \text{ \AA}$. The calculations were performed with SYBYL molecular modeling programme package (version.2.1) running on a Silicon Graphics Octane2 R12000 workstation. The structures were built manually in Sybyl followed by molecular mechanics geometry optimization using the Tripos force field with the conjugate gradient minimization method and a gradient of $0.01 \text{ kcal mol}^{-1} \text{ \AA}^{-1}$ as termination criterion. A stepwise regression has been done with the 96 descriptors obtained and the best 6 whose cumulative r^2 value reaches the 0.8 have been again involved together with the chosen Dragon descriptors into a final stepwise regression analysis.

Results and Discussion

For the easier understanding and convenience the molecular structure of the azole derivatives has been divided into four component blocks (*Fig. 1*). Within blocks the following structural modifications have been carried out.

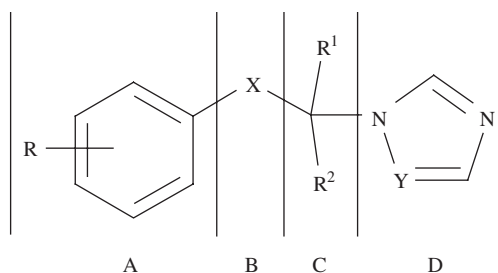


Fig. 1. The general structure and the component blocks of the azole fungicides.
For the substituents and the individual compounds see *Table 1*

Block A: Introduction of various groups in the para, meta and ortho positions of the phenyl group;

Block B: Change in the oxidation state (carbonyl or hydroxyl function) and substitution of the hydroxyl group to chlorine atom, involving change also in geometry (sp^3 or sp^2 hybridization) of the carbon atom;

Block C: Alkyl substitution on the carbon adjacent to the azole ring;

Block D: The placement of an imidazole or 1,2,4-triazole ring.

First consider Block B. The reduction of the carbonyl function to carbinol derivative decreases the AAA to a great extent in almost all cases of the imidazole derivatives (**2**, **9**, **15**, **21**, **23**, **33**, **41**, **45**, **47**) except 2,4-dichloro derivative **40** the reduction of which results in an increase to a small extent (**41**). The reversed situation can be observed in the case of the keto derivatives ($X = C=O$) of the 1,2,4-triazole compounds. Their poor activity is enhanced to a small degree or unchanged by the reduction to carbinol derivatives ($X = CH-OH$, **6**, **12**, **17**, **29**). The reduction of the carbonyl function has three simultaneous consequences. The appearance of a more polar and protic hydroxyl group which is capable to form hydrogen bond by acceptance and donation alike, while the carbonyl group can only accept hydrogen, as well as the change of the geometry of the carbon atom, regarding sp^3 or sp^2 hybridization. The latest seems to have the lesser influence on efficacy as the acetylation of the hydroxyl group of **2** to **4** results in a more than two times enhancement in the activity while both compounds have the same geometry. On the other hand, the substitution of the hydroxyl group to chlorine atom (**3**, **7**, **10**, **13**, **27**, **31**, **34**, **37**, **39**, **42**, **43**) causes a dramatic activity increase whereas this substitution does not change the geometry. The *p*-benzyloxy substituent (**48–54**) has activity enhancing effect.

Within block C, the examination of the influence of the alkyl substituents on the activity shows that omitting both methyl groups at the keto-derivatives ($X = C=O$) leads to a decline in the activity (see **1–14**, **5–16** and **25–35** compound pairs). Substantial improvement can be achieved by lengthening the methyl group of **8** and **26** to an ethyl (**18** and **30**) as well as to an *i*-butyl (**19**) and a *n*-butyl group (**32**), respectively. In the case of the 1-chloro derivative **27** the lengthening only to an ethyl group yields a compound (**31**) with similar activity, but to a *n*-butyl group results in a derivative (**34**) the activity of which is about only the half of that of **27**. The activity increasing effect of the longer alkyl group on the carbon atom adjacent to the azole ring can be explained with their property enhancing the lipophilicity. On the other hand, this region can be important from the point of view of binding to the apoprotein (Yoshida and Aoyama, 1987; Guan et al., 1992). At the same time in the case of the 1-chloro-derivatives this cannot be an explanation. Possibly, these compounds act with different action mode(s), too as the keto derivatives have, and a steric hindrance of a longer chain can prevent the molecule from exerting the different activity for which the 1-chloro substituent may be responsible.

Most of the agricultural DMG fungicides contain 1,2,4-triazole ring. We do not yet know the explanation why the test compounds with 1,2,4-triazole ring have significantly weaker activity than the imidazole derivatives in our test system.

Investigating the role of the substituents in block A shows that a profound improvement in the potency can be achieved with the introduction of a *p*-chloro (**25**, **27**, **30**, **31**, **32**, **34**, **37**, **39**) and 2,4-dichloro (**40–43**) substituents. The activity increasing property of the chloro substituent is not surprising since many commercial azole fungicide (triadimefon, triadimenol, imazalyl, propiconazole, myclobutanil, paclobutrazol, prokloraz, tebukonazole) have chloro substituent(s) on their benzene ring.

The AAA of our test compounds as well as the reference fungicides have been evaluated with principal component analysis. For the grouping of test and reference compounds

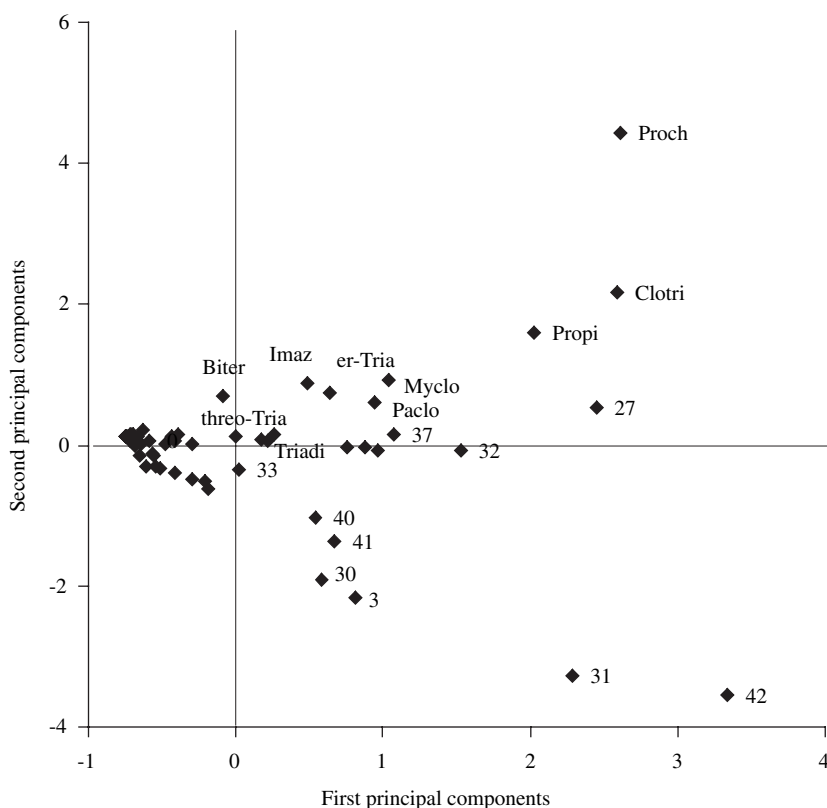


Fig. 2. The separation of the action modes of the 54 test compounds and the 10 reference fungicides by the first and second principal components calculated by the results of the tests on 30 fungal species

the first and the second principal components are used (Fig. 2). The two classes can be distinguished well from each other. Although the weight of the factors responsible for the separation is not too high within the total variance, but according to the second principal components the reference compounds take positive while the most of the test compounds have negative values. Two (31, 42) of our three most effective compounds (27, 31, 42) form a well separating group and are situated farthest distance to the reference compounds (see Fig. 2). Together with the reasons described above, other mode of actions can be also assumed to be responsible for the antifungal activity of these two compounds to those which are responsible for the action of the commercial fungicides tested.

The first principal components, obtained by considering the data of only the test compounds, explain the 70 percent of the variance and show high correlation ($r^2 = 0.97$) with the AAA.

To find the chemical and physico-chemical properties influencing the AAA of our compounds two series of molecular descriptors have been calculated applying the Dragon

as well as the Sybyl VolSurf programs. With the procedure described in the “Materials and Methods” an equation with five variables has been obtained.

$$\begin{aligned} \text{AAA (\%)} (\pm 4.02) = & -39.33 + 837.35 (\pm 130.81) * \text{X4Av} - 1.45 (\pm 0.91) * \text{HL1} + \\ & + 12.31 (\pm 4.06) * \text{H3m} - 0.60 (\pm 0.40) * \text{RDF060m} + 1.12 (\pm 1.09) * \text{Iw7} \\ N = 54, r^2 = 0.80, F_{(5,48)} = 38.54 \end{aligned}$$

This equation contains Dragon and VolSurf descriptors alike among which the three Dragon descriptors (X4Av, H3m and RDF060m) represent those properties responsible for the AAA to a greater extent than to those of VolSurf descriptors.

X4Av, belonging to the topological descriptors, is an average valence connectivity index $X-4$. Topological indices are related either to a vertex adjacency relationship (atom-atom connectivity) in the graph (molecular structure) G or to topological distances in G . Topological indices based on the atom-atom connectivity are usually formulated as the total sum of some combination of degrees of the adjacent vertices (atoms).

H3m is a member of the newly discovered GATEWAY (GEometry, Topology, and Atom-Weights Assembly) descriptors (Consonni et al., 2002), based on a leverage matrix called *Molecular Influence Matrix*, which are a new molecular representation easily calculated from the spatial coordinates of the molecule atoms in a chosen conformation. They try to match 3D-molecular geometry provided by the molecular influence matrix and atom relatedness by molecular topology, with chemical information by using different atomic weightings (atomic mass, polarizability, van der Waals volume, and electronegativity, together with unit weights). H3m is weighted by atomic masses.

RDF060m (Hemmer et al., 1999) is the representation of the 3D structure of a molecule by a radial distribution function RDF code, which is obtained with the simulation of an infrared spectrum by a counterpropagation CPG neural network.

An important prerequisite for a pesticide (and drug) to be active is that it is able to reach its site of action. The preferred and most widely used route of pesticide administration is their application on the surface of the target pest. In the penetration from the surface inside the pest the passive diffusion takes part to a great extent. This process depends heavily on the molecule's ability to diffuse through the lipophilic membranes. In turn, the diffusion depends on the solvation desolvation processes, on the surface's interaction between solute and membrane, and on the H-bond potential of the solute. The aim of *in silico* procedures is to be able to predict the penetration properties of pesticide and drug candidates. This task is conducted by VolSurf program which produces and explores the physico-chemical property space of a molecule (or library of molecules) starting from 3D maps of interaction energies between the molecule and chemical probes. The basic concept of VolSurf is to compress the information present in 3D grid maps into few 2D numerical descriptors (Cruciani et al., 2000) which are simple to understand and to interpret.

HL1, representing the hydrophilic-lipophilic balance, is the ratio between the hydrophilic regions measured at -3 kcal/mol and the hydrophobic regions measured at -0.6 kcal/mol. The balance describes which effect dominates in the molecule, or if they are roughly equal balanced.

Iw7 is an integrity moment which is calculated at energy levels of 5.0 kcal/mol and measures the unbalance between the center of mass of a molecule and the position of the hydrophilic regions around it. Integrity moment is vector pointing from the center of mass to the center of Iw7. If the integrity moment is high, the hydrated regions are clearly concentrated in only one part of the molecular surface. If the integrity moment is small, the polar moieties are either close to the center of mass or at opposite ends of the molecule; thereby, the resulting barycenter is close to the center of the molecule.

From the equation above it is obvious that the factors describing the chemical structure itself (Dragon descriptors) have greater influence on the AAA than the factors which are responsible for the passive penetration (VolSurf descriptors).

In conclusion among the 54 azole derivatives synthesized there are more the fungicidal activity of which are equipotent in the tests applied by us with the highly active commercial fungicides. The 1-chloro-derivatives, beyond that, possess more action modes, one of which is different to the demethylase inhibition. The development of resistance against molecules bearing more action sites is slower than those acting with only one mode (Pommer, 1987). On the bases of these all we are hopeful that some of our compounds can be lead in the development of effective new fungicide with broad spectrum.

Acknowledgements

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Literature

- Bélai, I., Matolcsy, Gy., Farnsworth, D. E. and Feyereisen, R. (1988): Inhibition of insect Cytochrome P-450 by some metyrapone analogues and compounds containing a cyclopropylamine moiety and their evaluation as inhibitors of juvenile hormone biosynthesis, *Pestic. Sci.* 24, 205–219.
- Bélai, I., Darvas, B., Bauer, K. and Tag El-Din, M. H. (1995): Effects of anti-ecdysteroid azole analogues of metyrapone on the larval development of the fleshfly, *Neobellieria bullata*. *Pestic. Sci.* 44, 225–232.
- Brown, I. F., Jr., Taylor, H. M. and Hackler, R. E. (1984): Synthesis-directing structure-activity relationships of some fungicides inhibiting ergosterol biosynthesis. In: P. S. Magee, G. K. Kohn and J. J. Menn (eds): *Pesticide Synthesis Through Rational Approaches*. ACS Symp. Ser. 225), American Chemical Society, Washington, pp. 65–82.
- Buchenauer, H. (1977): Mode of action and selectivity of fungicides which interfere with ergosterol biosynthesis. In: *Proc. Brit. Crop. Conf.* Brighton, pp. 699.
- Consonni, V., Todeschini, R. and Pavan, M. (2002): Structure/Response correlations and similarity/diversity analysis by GETAWAY Descriptors. 1. Theory of the Novel 3D Molecular Descriptors. *J. Chem. Inf. Comput. Sci.* 42, 682–692.
- Cruciani, G., Crivori, P., Carrupt, P.-A. and Testa, B. (2000): Molecular fields in quantitative structure–permeation relationships: the VolSurf approach. *J. Molec. Struct. (Theochem)* 503, 17–30.
- Darvas, B., Rees, H. H., Hoggard, N., Tag El-Din, M. H., Kuwano, E., Bélai, I. and Timár, T. (1992): Cytochrome P-450 inducers and inhibitors interfering with ecdysone 20-monooxygenases and their activities during postembryonic development of *Neobellieria bullata* Parker. *Pestic. Sci.* 36, 135–142.

- Fujimoto, T. D., Quinn, J. A., Egan, A. R., Shaber, S. H. and Ronald, R. R. (1988): Quantitative structure-activity relationship studies of the fungitoxic properties of phenethyl 1,2,4-triazoles. *Pest. Biochem. Physiol.* 30, 199–213.
- Gadher, P., Mercer, E. J., Baldwin, R. C. and Wiggins, T. E. (1983): A comparison of the potency of some fungicides as inhibitors of sterol 14-demethylation. *Pest. Biochem. Physiol.* 19, 1–10.
- Guan, J., Braks, H. M. J., Kerkenaar, A. and De Waard, M. A. (1992): Interaction of microsomal cytochrome P450 isozymes isolated from *Penicillium italicum* with DMI fungicides. *Pestic. Biochem. Physiol.* 42, 24–34.
- Hawksworth, D. L., Kirk, P. M., Sutton, B. C. and Pegler, D. N. (1995): Ainsworth and Bisby's Dictionary of the Fungi, 8th Edition, CAB International, Wallingford, UK, pp. 1–616.
- Hemmer, M. C., Steinhauer, V. and Gasteiger, J. (1999): Deriving the 3D structure of organic molecules from their infrared spectra. *Vibrational Spectroscopy* 19, 151–164.
- HyperChem 5.1 for Windows Molecular Modeling System, Hypercube, Inc., Gainesville, Florida, 1997.
- Marchington, A. F. (1984): The design of triazole fungicides. In: Magee, P. S., Kohn, G. K. and Menn, J. J. (eds): *Pesticide Synthesis Through Rational Approaches*. ACS Symp. Ser. 225, American Chemical Society, Washington, pp. 173–183.
- Nakayama, A. (1992): Quantitative structure-activity relationships and molecular graphics study of triflumizole and related fungicidal imidazoles. In: W. Draber and T. Fujita (eds): *Rational Approaches to Structure, Activity and Ecotoxicology of Agrochemicals*. CRC Press, London, pp. 445–463.
- Oros, G., Cserhádi, T. and Forgács, E. (2002): Antifungal activity of some trityl-based synthetic dyes. *Environmental Toxicology and Chemistry* 21, 1206–1212.
- Parberry, D. G. (1996): Trophism and ecology of fungi associated with plants. *Biol. Rev.* 71, 473–527.
- Podust, L. M., Poulos, T. L. and Waterman, M. R. (2001): Crystal structure of cytochrome P450 14 α -sterol demethylase (CYP51) from *Mycobacterium tuberculosis* in complex with azole inhibitors. *PNAS* 98, 3068–3073.
- Pommer, E. H. (1987): Morpholine Fungicides. In: H. Lyr (ed.): *Modern Selective Fungicides*. VEB Gustav Fischer Verlag, Jena, pp. 143–157.
- Todeschini, R. and Consonni, V. (2000): *Handbook of Molecular Descriptors, Methods and Principles in Medicinal Chemistry* Vol. 11; Wiley-VCH: Weinheim.
- Todeschini, R., Consonni, V. and Pavan, M. (2005): DRAGON. Software for the calculation of molecular descriptors, version 5, available in the WEB: <http://www.disat.unimib.it/chm>
- Van den Bossche, H. (1985): Biochemical targets for antifungal azole derivatives: Hypothesis on the mode of action. In: McGinnis, M. R. (ed.): *Current Topics In: Medicinal Mycology*. Springer-Verlag, Berlin, Vol. 1, pp. 313–315.
- Vanbreuseghen, R., Cutsem, I. V. and Thienpot, D. (1967): Experimental study of a new fungistatic agent: RI0100 (1-(1,2,3,4-Tetrahydro-1-naphthyl)-5-(ethoxycarbonyl)imidazole nitrate). *Chemotherapia* 12, 107–122.
- Yoshida, Y. and Aoyama, Y. (1987): Interaction of azole antifungal agents with cytochrome P-450_{14DM} purified from *Saccharomyces cerevisiae* microsomes. *Biochem. Pharmacol.* 36, 229–235.