



A MOLECULAR DOCKING ANALYSIS ASSESSING THE POSSIBLE ENANTIOSELECTIVITY OF THE INTERACTIONS OF FLUTRIAFOL STEREOISOMERS WITH SOIL ENZYMES

Diana-Larisa Roman, Adriana Isvoran

Department of Biology-Chemistry, West University of Timisoara, 16 Pestalozzi, 300115 Timisoara, Romania and Advanced Environmental Research Laboratories, West University of Timisoara, Oituz 4, Timisoara, Timiș, 300086, Romania

Abstract: Flutriafol, with the IUPAC name 1-(2-fluorophenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl)ethanol, is a systemic fungicide that is applied to combat diseases in many types of crops, turf, and ornamental plants. Flutriafol has one chiral carbon and two enantiomers, (R)- and (S)-flutriafol. Specific scientific literature contains little information regarding the environmental effects of the enantiomers of flutriafol. It was shown that, usually, the (R) isomer reveals greater activity against some fungal species and also greater acute toxicity to earthworms and algae. This study focuses on the implementation of molecular docking to evaluate the possible enantioselectivity of the interactions of the two stereoisomers of flutriafol with soil enzymes. Molecular docking study revealed that both enantiomers were able to bind to the active sites of dehydrogenases and phosphatases. The average value of the interaction energy observed for (R)-flutriafol with dehydrogenases was -7.85 kcal/mol, compared to -7.45 kcal/mol for the interaction of (S)-flutriafol with these enzymes. Similarly, the interaction energy obtained for the interaction of (R)-flutriafol with phosphatase was -9.16 kcal/mol, compared to -9.04 kcal/mol for the interaction of (S)-flutriafol with this enzyme.

• Introduction

Pesticides are extensively applied in agriculture to improve and maintain crop yields and control pests. Global population growth is the main factor leading to an increasing use of pesticides to provide food for the entire population. For almost all types of agricultural crops, pesticides are often used several times a year, and the high use of pesticides results in the storage of residues in the soil, especially in groundwater, affecting ecosystems. To prevent or eradicate fungal infections in crops, fungicides belonging to the azoles and triazoles group have been frequently used.

• Results and discussions

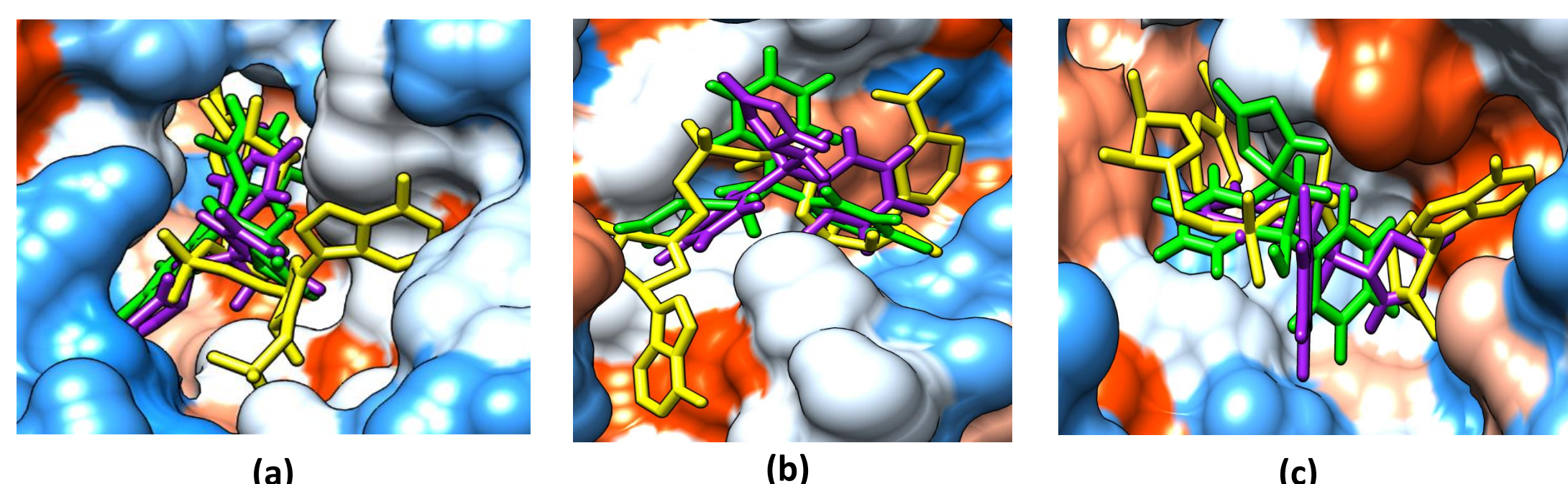


FIG 2. Binding of (R)-flutriafol (purple sticks) and of (S)-flutriafol (green sticks) to the active site of dehydrogenases belonging to *Clostridium beijerinckii* (a), *Aspergillus fumigatus* (b) and *Rhizobium leguminosarum* (c). Dehydrogenases are represented as solid hydrophobicity surfaces, blue regions being hydrophilic and orange regions being hydrophobic. The substrates (yellow sticks) dihydronicotinamide-adenine-dinucleotide phosphate for *Clostridium beijerinckii* dehydrogenase (a) and 1,4-dihydro-nicotinamide-adenine-dinucleotide for *Aspergillus fumigatus* (b) and *Rhizobium leguminosarum* illustrate the position of the active site.

Table 1. Interaction energies for the binding of the flutriafol enantiomers to soil enzymes

Enzyme	Organism	ΔG (kcal/mol)	
		(R)-flutriafol	(S)-flutriafol
Dehydrogenase	<i>Clostridium beijerinckii</i>	-8.41	-8.48
	<i>Aspergillus fumigatus</i>	-7.51	-6.89
	<i>Rhizobium leguminosarum</i>	-7.63	-7.38
Phosphatase	<i>Bacillus subtilis</i>	-7.54	-7.75
	<i>Aspergillus niger</i>	-7.22	it does not bind to the active site
	<i>Rhizobium leguminosarum</i>	-7.32	-7.16
Catalase	<i>Komagataella pastoris</i>	-9.16	-9.04

• Conclusions

- molecular docking analysis highlighted distinct interaction energies between the flutriafol enantiomers and soil dehydrogenases, phosphatases, and one of the catalases, with (R)-flutriafol providing higher interaction energies;
- distinct spatial orientations of the 2 enantiomers in the catalytic sites of these enzymes were also observed; these findings emphasize the distinct bioactivity of flutriafol enantiomers and the need to use the most active stereoisomer against fungi in practice so as to maximize efficacy and minimize the side effects of the fungicide;
- to our knowledge, this is the first study on the interactions of flutriafol enantiomers with enzymes found in soil;
- in our opinion, understanding the distinct bioactivities of fungicide enantiomers is important for the development of sustainable and ecological pest management strategies;
- monitoring the effect of fungicides, both individually and their enantiomers, on non-target ecosystems provides information on the toxicity and consequences of fungicide use.

• Material and method

Protein Data Bank;
AlphaFold Protein Structure Database;
Chimera software;
SwissDock online server;
Protein-Ligand Interaction Profiler.

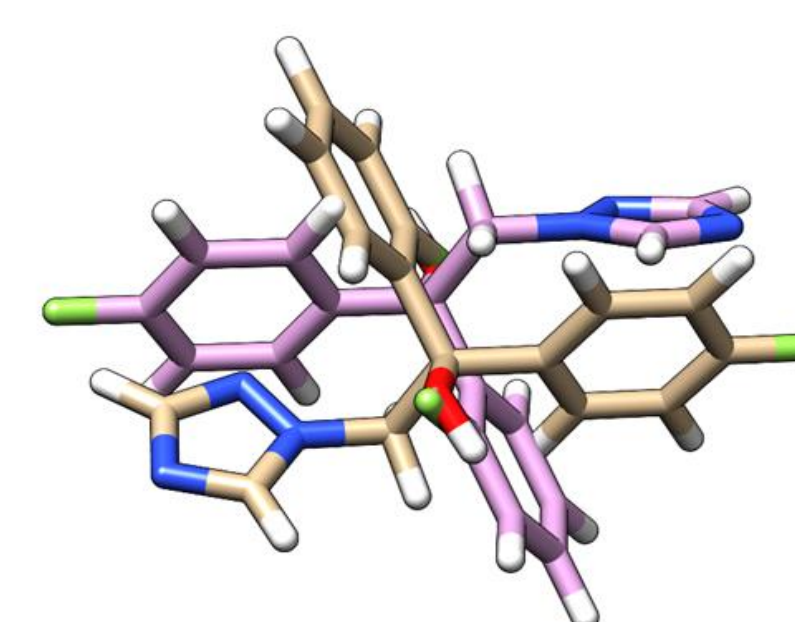


FIG 1. The structures of the two stereoisomers of flutriafol: (R)-flutriafol (brown carbon atoms) and (S)-flutriafol (purple carbon atoms). Oxygen atoms are colored red, nitrogen atoms are colored blue, hydrogen atoms are colored white and fluor atoms are colored green.

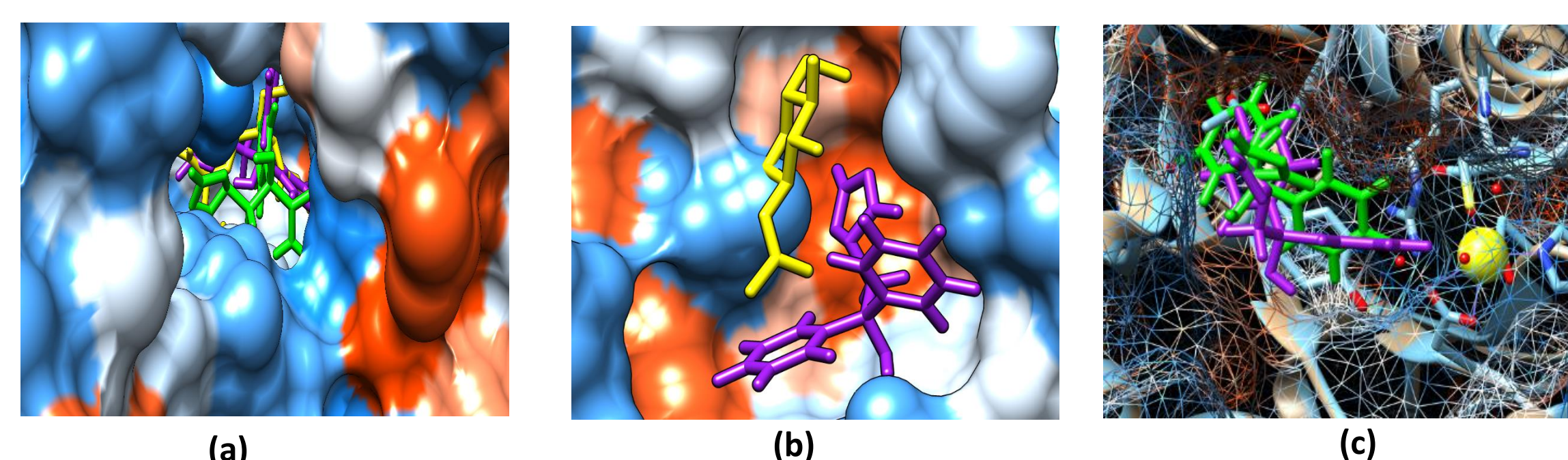


FIG 3. Binding of (R)-flutriafol (purple sticks) and of (S)-flutriafol (green sticks) to the active sites of phosphatases belonging to *Bacillus subtilis* (a), *Aspergillus niger* (b) and *Rhizobium leguminosarum* (c). *Bacillus subtilis* and *Aspergillus niger* phosphatases are represented as solid hydrophobicity surfaces and *Rhizobium leguminosarum* phosphatase is represented as mesh hydrophobicity surface, blue regions being hydrophilic and orange regions being hydrophobic. The position of the inhibitory peptide PhF (yellow sticks) illustrates the region of the active site of *Bacillus subtilis* phosphatase (a), the position of 2-acetamido-2-deoxy-beta-D-glucopyranose (yellow sticks) illustrates the region of the active site of *Aspergillus niger* phosphatase (b), and the calcium ion (yellow sphere) illustrates the active site region of *Rhizobium leguminosarum* phosphatase.

Table 2. Noncovalent interactions established by flutriafol enantiomers and residues of the enzymes considered in this study. For comparison, the noncovalent interactions for the substrates and enzyme residues are also presented: NADPH—1,4-dihydronicotinamide adenine dinucleotide, NAG-2-acetamido-2-deoxy-beta-D-glucopyranose.

Enzyme	Ligand	Residues involved in the non-covalent interactions
<i>Clostridium beijerinckii</i> dehydrogenase	(R)-flutriafol	GLY176, ALA177, VAL178, GLY179, ARG200
	(S)-flutriafol	THR38, ILE175, GLY176, ALA177, VAL178, TYR267, ILE345
	NADPH	THR38, SER39, ILE175, ALA176, VAL178, GLY179, SER199, ARG200, GLU247, ASN266, TYR267, LYS340
<i>Aspergillus fumigatus</i> dehydrogenase	(R)-flutriafol	GLY44, PHE45, THR139, GLN244
	(S)-flutriafol	GLY44, PHE45, THR137, THR139, GLU140
	NADPH	GLY44, PHE45, GLN74, ASP77 (2), ASP199, ASN200, VAL238, THR242
<i>Rhizobium leguminosarum</i> dehydrogenase	(R)-flutriafol	PRO161, TRP162, LYS186, SER240
	(S)-flutriafol	TRP162, LYS186, ALA188, SER240
	NADPH	THR160, PRO161, LYS186, PRO187, SER240, GLU261
<i>Bacillus subtilis</i> phosphatase	(R)-flutriafol	TYR66, TYR152, TYR153, ARG223, TYR226, LEU230, GLN263
	(S)-flutriafol	-
	Inhibitory peptide	TYR66, TYR152, TYR153, LYS155, GLN183, LEU187, ASP194, ARG223, TYR226, ASN227, SER260, GLN263, PHE266, TYR300, GLU303, ALA334, ASP335, ASP338
<i>Aspergillus niger</i> phosphatase	(R)-flutriafol	ALA292, ASN296, LEU415, ASP417, TYR418, THR419, LEU432
	NAG	ASN296, THR300, ASN439, LEU432
<i>Rhizobium leguminosarum</i> phosphatase	(R)-flutriafol	TYR105, PHE138, ASN141
	(S)-flutriafol	TYR105, LEU125, PHE138, ASN141, TYR215
	Calcium	ASP12, ASP324, HIS325

• References

- Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera—A Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* 2004, 25, 1605–1612.
- Roman, D.L.; Voiculescu, D.I.; Matica, M.A.; Baerle, V.; Filimon, M.N.; Ostafe, V.; Isvoran, A. Assessment of the Effects of Triticonazole on Soil and Human Health. *Molecules* 2022, 27, 6554.
- Roman, D.L.; Voiculescu, D.I.; Filip, M.; Ostafe, V.; Isvoran, A. Effects of Triazole Fungicides on Soil Microbiota and on the Activities of Enzymes Found in Soil: A Review. *Agriculture* 2021, 11, 893.

Acknowledgement: This work was supported by the grant PN-III-P1-1.1-PD-2019-0255 "Assessment of the environmental effects of triazole fungicides that are approved for use in European Union for the protection of cereals".