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New Perspective on Comparative Chemometric and Molecular Modeling of Antifungal Activity and Herbicidal Potential of Alkyl and Cycloalkyl *s*-Triazine Derivatives

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Abstract: The contamination of the environment by pesticides is becoming a burning issue in many countries in the World. Development, design, and synthesis of new eco-friendly pesticides and modification of existing ones in order to improve their efficacy with the lowest impact on the environment are two main future possibilities in crop protection and the provision of sufficient food for the growing world population. The present study is focused on the comparative analysis of a series of eight symmetrical triazine derivatives, as potential herbicide candidates with acyclic (alkyl) and cyclic (cycloalkyl) substituents, in terms of their antifungal activity towards *Aspergillus flavus* as an opportunistic fungal pathogenic microorganism responsible for frequent contaminations of crops with aflatoxin, and in terms of their potential application as herbicides in maize, common wheat, barley, and rice crops. The applied methods include the chemometric pattern recognition method (hierarchical cluster analysis), experimental microbiological analysis of antifungal activity (agar well-diffusion method), and molecular docking of the triazines in the corresponding enzymes. The main findings of the conducted study indicate the significant antifungal activity of the studied triazine derivatives towards *A. flavus*, particularly the compounds with acyclic substituents; five out of eight studied triazines could be applied as systematic herbicides, while the other three triazines could be used as contact herbicides; the compounds with acyclic substituents could be more suitable for application for various crops protection than triazines with cyclic substituents.

Keywords: chemometrics; molecular modeling; molecular docking; pesticides; *Aspergillus flavus*; triazines

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1. Introduction

Considering today's pesticides with limited efficiency and significant toxicity, and the fact that plant protection must be efficient enough, the balance between the maximal yield of crops and minimal toxicity on the environment and living organisms is a prime concern in modern agriculture. There are numerous studies that deal with the development of novel pesticides that will be sufficiently effective in plant protection with minimal environmental hazard, as well as minimal toxic effects on human and animal health [1–3]. Moreover, taking into account the fact that World's population reached 8 billion people in 2022, crop yields will have to be raised in the near future in order to prevent the destruction of wildlands and the starvation of millions of people [4]. When misused, numerous synthetic pesticides have a high potential to eliminate beneficial organisms, such as earthworms, bees, etc., and can cause serious water and soil pollution, potentially causing pest resistance [5]. Nevertheless, the use of synthetic pesticides in agriculture is still common worldwide for achieving one of today's top priorities: providing enough food for the growing World population.

Triazine derivatives are a commonly used group of nitrogen-containing compounds for weed control in crop production [6–8]. Some triazine derivatives express antifungal activity towards many fungi including *Cryptococcus neoformans*, *Aspergillus niger*, *Candida albicans*, *Colletotrichum capsici*, etc. [9–13]. However, triazines can be degraded under biotic

and abiotic processes forming potentially toxic metabolic products. Some microorganisms have a high potential of biodegradation of triazine derivatives, such as *Phanerochaete chrysosporium* (atrazine), *Variovorax sp*. (simazine), *Arthrobacter nicotinovorans* (atrazine), *Penicillium sp*. (simazine), *Aspergillus sp*. (atrazine, simazine, propazine), etc. [14]. One of the most used triazine herbicides is atrazine which can be accumulated in the environment representing a threat to living organisms, especially due to its high mutagenic and carcinogenic potential [15].

Considering all the problems (environmental and health hazards) with the existing pesticides, whose application is permitted worldwide, there are many ongoing studies that are focused on modification of the existing pesticide structures by adding new substituents and/or removing the existing ones or changing the crucial molecular features to make new eco-friendly, efficient, low-cost and safer pesticides. Computational chemistry, including the quantitative structure–property relationship (QSPR) and quantitative structure–activity relationship (QSAR) approaches, provides precious tools for the search for novel bioactive compounds. Combined with chemometric pattern recognition and regression methods, QSPR and QSAR approaches extract significant information from the pool of data and enable the researchers to predict the physicochemical properties and biological activity of novel compounds. Molecular docking and QSAR method were applied in the modeling of 4-anilinoquinoline-triazine hybrids as pf-DHFR inhibitors [16], as well as computer-aided molecular modeling (CAMM) of mechanisms of the antigen–antibody interaction for a set of triazine herbicides [17]. QSAR with chemometric techniques was successfully employed for the prediction of the toxicity of a set of triazine compounds [15,18]. Some studies were focused on the QSAR analysis of the binding of triazines with mono- and polyclonal antibodies [19] and on the analysis of triazine derivatives as novel h-DAAO inhibitors by 3D-QSAR, molecular dynamics simulations, and molecular docking approaches [20].

Another aspect of the analysis of pesticides is related to the determination of their lipophilicity by applying experimental and in silico approaches. The experimental approaches include the classical shake-flask method; however, nowadays, reversed-phase thin-layer chromatography (RP-TLC) and reversed-phase high-performance liquid chromatography (RP-HPLC) are the most common methods for lipophilicity determination of pesticides [21–23]. In silico methods provide numerous calculation procedures to estimate the lipophilicity of various groups of compounds expressed as the logarithm of the partition coefficient (logP). Since different computational programs utilize different calculation procedures, the logP values of the same compound may vary, therefore the consensus (average) logP parameter is usually the most appropriate in silico descriptor of lipophilicity. Considering the herbicides (or pesticides, in general), lipophilicity is the key parameter in their classification as systemic or non-systemic (contact) herbicides [24].

Bearing in mind the importance, applications, advantages, and disadvantages of the currently used triazine derivatives in agriculture, development, design, and synthesis of new compounds is desirable. The present study is focused on: (1) the analysis and comparison of a synthesized but unaffirmed group of symmetrical triazine derivatives (*s*-triazines) with acyclic and cyclic substituents (2-chloro-4,6-alkylated diamino-*s*-triazines) with affirmed commercial triazine and non-triazine herbicides; (2) estimation of their potential application as systemic or contact herbicides; (3) determination of their antifungal activity towards *A. flavus* as the most prevalent fungus responsible for frequent aflatoxin contaminations of crops, molecular docking analysis of the analyzed compounds on correspondent enzymes of *A. flavus* fungus; and (4) the prediction and comparative analysis of the binding affinity of the series of *s*-triazines towards proteins of several crops using molecular docking approach.

2. Materials and Methods

2.1. The Series of the Analyzed 2-Chloro-4,6-Alkylated Diamino-S-Triazines

The molecular structures of the studied compounds are presented in Figure 1. The compounds were synthesized at the Faculty of Technology and Metallurgy, University of

Belgrade, according to the procedure described earlier [25,26]. Their IUPAC names and the SMILES codes are presented in Table 1.

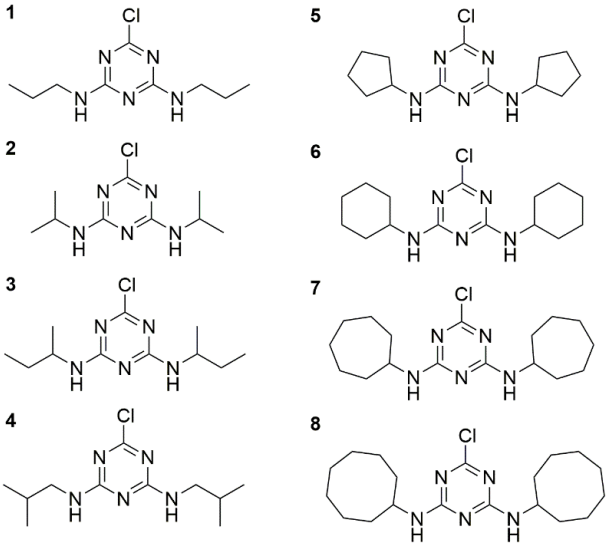

Figure 1. Molecular structures of the studied *s*-triazines (**1**–**8**).

Table 1. The IUPAC names and the SMILES codes of the studied triazines.

Comp.	IUPAC Name	SMILES Codes
1	6-chloro-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine	ClC1=NC(NCCC)=NC(NCCC)=N1
2	6-chloro-N^2,N^4-diisopropyl-1,3,5-triazine-2,4-diamine	ClC1=NC(NC(C)C)=NC(NC(C)C)=N1
3	N^2,N^4-di-sec-butyl-6-chloro-1,3,5-triazine-2,4-diamine	ClC1=NC(NC(C)CC)=NC(NC(C)CC)=N1
4	6-chloro-N^2,N^4-diisobutyl-1,3,5-triazine-2,4-diamine	ClC1=NC(NCC(C)C)=NC(NCC(C)C)=N1
5	6-chloro-N^2,N^4-dicyclopentyl-1,3,5-triazine-2,4-diamine	ClC1=NC(NC2CCCC2)=NC(NC3CCCC3)=N1
6	6-chloro-N^2,N^4-dicyclohexyl-1,3,5-triazine-2,4-diamine	ClC1=NC(NC2CCCCC2)=NC(NC3CCCCC3)=N1
7	6-chloro-N^2,N^4-dicycloheptyl-1,3,5-triazine-2,4-diamine	ClC1=NC(NC2CCCCCC2)=NC(NC3CCCCCC3)=N1
8	6-chloro-N^2,N^4-dicyclooctyl-1,3,5-triazine-2,4-diamine	ClC1=NC(NC2CCCCCCC2)=NC(NC3CCCCCCC3)=N1

The studied compounds belong to the group of symmetrical triazines having the same substituents on N^2 and N^4 atoms. The compounds are generally named 2-chloro-4,6-alkylated diamino-*s*-triazines. Compounds **1**–**4** possess acyclic alkyl substituents, while compounds **5**–**8** have cycloalkyl substituents. Compounds **1** and **2**, as well as compounds **3** and **4**, are structural isomers, while compounds **5**–**8** are members of the homologous series in which the rings differ in a methylene group. The selection of the compounds of interest was carried out based on their structural characteristics, solubility, and stability. Moreover, the structures were selected with the intention of seeing how structural isomerism influences the mobility of the compounds as well as their antifungal activity. Among the analyzed compounds, there is a compound commercially known as propazine (compound **2**). Compound **1** is structurally very similar to simazine (6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine), which is a well-known commercial herbicide.

2.2. The Calculation of Molecular Descriptors and Comparative Analysis based on Bromilow's Approach. Hierarchical Clustering

The molecular descriptors taken into account in the present study are the in silico lipophilicity descriptors and pK_a values. The lipophilicity descriptors were calculated earlier [27] by different programs, including ALOGPS 2.1, MarvinSketch 14.9.15.0, $SWISS_{ADME}$, and ChemBioDraw 13.0, based on which the consensus logP values were calculated. The logP parameters of additional (commercial) triazines were calculated using the same procedure as in the case of compounds **1**–**8**. The calculations were based on 2D molecular structures.

Generally, pesticides with low polarizability and low molecular weight possess high volatilization potential [28]. The polarizability of the analyzed compounds was calculated by the MarvinSketch program 14.9.15.0.

Bromilow's approach, introduced by Bromilow and co-workers [24], takes into account the lipophilicity (logP) and acidity (pK_a) of herbicides in order to predict whether they will be transported by phloem, the living tissue in vascular plants that transports organic compounds made in photosynthesis to other parts of the plant (translocation), or by xylem, which is responsible for transporting water and nutrients from roots to leaves and stems [24]. Bromilow's model is useful for predicting herbicide mobility in the plant at physiological pH. The classification of systemic and non-systemic pesticides is based on the threshold of logP = 4 [24]. Systemic pesticides are characterized by logP < 4, and non-systemic pesticides by logP > 4 (highly lipophilic compounds). Besides lipophilicity, the acidity of the compounds has an influence on their transport in the plant. More acidic compounds will be transported by phloem, while the compounds that have low acidity ($pK_a > 6$) will be only transported by xylem [24].

In order to compare the lipophilicity and the type of transport of the analyzed compounds with commercially available triazine herbicides such as atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine) and simazine (6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine) and non-triazine herbicides including dicamba (3,6-dichloro-2-methoxybenzoic acid) and glyphosate (*N*-(phosphonomethyl)glycine) (Figure 2), the hierarchical cluster analysis (HCA) was applied. The HCA was based on Ward's algorithm and Euclidean distances. The HCA was carried out on real data (without normalization since all the data were on the same scale).

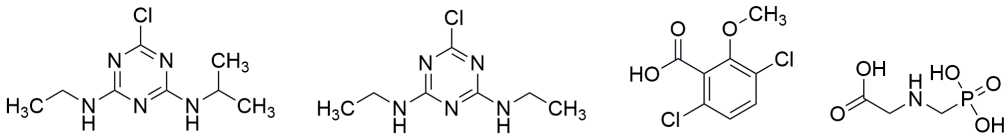

Figure 2. The molecular structures of the commercial herbicides considered in the comparative analysis.

2.3. Molecular Docking Analysis of the Affinity of Studied Compounds towards Cereals Proteins

The comparative molecular docking analysis was carried out in order to estimate the affinity towards the enzymes of common cereals of the analyzed *s*-triazines in comparison with the commercial herbicides. The corresponding proteins were prepared for analysis, so the non-relevant chains and water molecules were removed, and polar H-atoms and Kollman charges were added. A grid box was set for each dimension at 25 Å and captured the active site of a protein. Prior to the molecular docking, the molecular structures of the ligands were structurally optimized and converted into PDBQT format. Afterwards, the multiligand docking analysis was performed using AutoDock Vina software [29,30]. The groups of proteins, their codes, and cereals used in the analysis are listed in Table 2. The protein structures were retrieved from the Protein Data Bank (PDB) database (https://www.rcsb.org/, accessed on 24 November 2022).

2.4. Microbiological Analysis of Antifungal Activity and Molecular Docking Analysis

Antifungal activity testing was performed using the test strain *Aspergillus flavus* PA2D SS. A suspension of the test microorganism was prepared using 7-day-old culture and sterile saline to achieve 10^5 spores/mL. Melted and tempered (50 ± 1 °C) SMA (Sabouraud maltose agar) medium (15 mL, Himedia Laboratories, India) was inoculated using 1 mL of the pathogen suspension and poured into the Petri dish. After medium solidification, three wells per plate with a diameter of 10 mm were made. Triazine samples were dissolved in dimethylsulfoxide (DMSO, AlfaAesar, Germany) at a concentration of 10 mg/mL and tested in triplicate (3 × 100 µL). Incubation was carried out at 26 °C for 7 days, followed by

measurements of the inhibition zone diameters. The negative control was sterile distilled water applied instead of triazine samples.

Table 2. The groups of proteins, protein PDB codes, and cereals considered in the molecular docking analysis.

No.	Cereals	Protein PDB Code	PDB Protein Classification
1	*Zea mays* (maize)	4KPO	Hydrolases
2	*Zea mays*	1HXJ	Hydrolases
3	*Zea mays*	5ZJI	Oxidoreductases
4	*Zea mays*	4PXN	Oxidoreductases
5	*Zea mays*	7UBU	Transferases
6	*Zea mays*	1AXD	Transferases
7	*Hordeum vulgare* (barley)	1AVA	Hydrolases
8	*Hordeum vulgare*	1BG9	Hydrolases
9	*Hordeum vulgare*	2BGQ	Oxidoreductases
10	*Hordeum vulgare*	2VDG	Oxidoreductases
11	*Hordeum vulgare*	4HLN	Transferases
12	*Hordeum vulgare*	3TCM	Transferases
13	*Triticum aestivum* (common wheat)	3SC2	Hydrolases
14	*Triticum aestivum*	6GER	Hydrolases
15	*Triticum aestivum*	3AIR	Hydrolases
16	*Triticum aestivum*	5TTE	Ligases
17	*Triticum aestivum*	6NYA	Transferases/Ligases
18	*Triticum aestivum*	1WHT	Serine carboxypeptidases
19	*Oryza sativa* (rice)	5ZCL	Plant protein
20	*Oryza sativa*	2WG8	Hydrolases
21	*Oryza sativa*	3PTK	Hydrolases
22	*Oryza sativa*	2CVO	Oxidoreductases
23	*Oryza sativa*	4KVL	Oxidoreductases
24	*Oryza sativa*	1PKU	Transferases

In order to gain an overview of molecular interactions of the studied series of *s*-triazines with the corresponding enzymes of *A. flavus*, and to provide an explanation of the resulting antifungal activity, the molecular docking analysis was performed in the way already described in Section 2.3. The docking analysis was performed on the proteins retrieved from the PDB database (https://www.rcsb.org/, accessed on 24 November 2022) including: oxidoreductases (1R56, 1WS2, 1WS3, 1XXJ, 1XY3, 2PES, 4YNT, 4YNU, 5J7X), transferases (5ZZD, 6INW, 6IV7, 6J24, 6J46, 6JOH, 6J1O, 6K3H), antifungal proteins/inhibitors (6DRS, 6DTC), biosynthetic proteins (6IX3, 6IX8, 7WGH, 7WGI), and flavoprotein (6Y48).

3. Results and Discussion

3.1. Comparative Analysis Based on Lipophilicity and Bromilow's Model

One of the most important molecular features that dictate the fate of a compound in the environment and in a living organism is lipophilicity. Herbicides and other types of pesticides interact not only with the target (plants or insects) but also with various microorganisms. The distribution, translocation, and efficacy of pesticides are strongly influenced by their lipophilicity. The in silico lipophilicity parameters of the studied triazines were calculated using various programs [27]. A consensus logP (average lipophilicity) was selected for the general lipophilicity comparison of the analyzed triazines.

In order to compare the studied triazines with the triazines and non-triazines that are commercially available, several commercial herbicides were included in the comparative analysis. Their in silico lipophilicity descriptors are presented in Table A1 in the Appendix A. Their structures are generally similar to the structures of the triazines with acyclic substituents (compounds **1**–**4**), particularly atrazine and simazine, which are structurally very similar to compounds **1** and **2** which are structural isomers. The dendrogram,

which is based on the lipophilicity values of studied triazines and commercial herbicides, is presented in Figure 3.

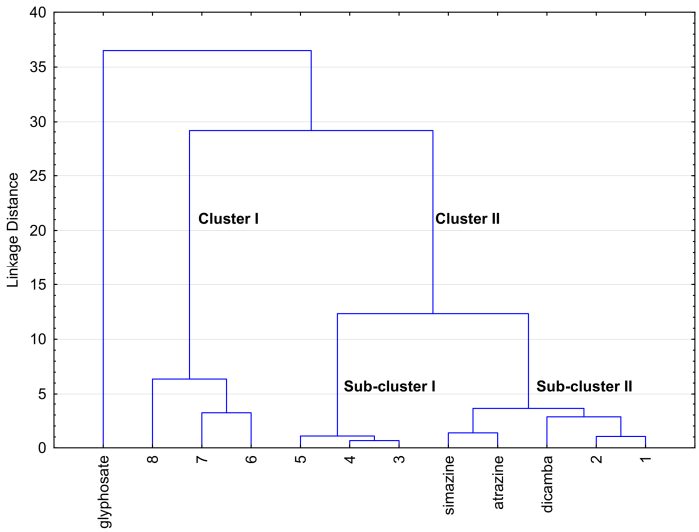

Figure 3. The dendrogram of the hierarchical clustering of the studied triazines and commercial herbicides based on in silico lipophilicity parameters.

Hierarchical clustering indicated the separation of the compounds into two main clusters. Cluster I contains only three triazines with cyclic substituents: cyclohexyl, cycloheptyl, and cyclooctyl, which are highly lipophilic compounds with no resemblance in terms of lipophilicity with the commercial ones. The second cluster is divided into two sub-clusters, so sub-cluster I contains compounds **3** and **4** (structural isomers with acyclic substituents) and compound **5** (triazine with cyclopentyl substituents). Regardless of its cyclic structure, compound **5** is closer to compounds **3** and **4** in the space of the considered lipophilicity parameters than to the other cycloalkyl triazines from the series. Sub-cluster II includes compounds **1** and **2** together with dicamba, simazine, and atrazine. Those compounds have the lowest lipophilicity and similar logP values. Glyphosate, which is a compound that is structurally most different from the others, can be considered an outlier since it does not belong to any cluster and is significantly separated from the others, which can be noticed in the dendrogram in Figure 3.

The obtained results indicated which analyzed compounds could be used as systemic or contact herbicides; however, in order to confirm the assumptions, more parameters are needed. Hence, Bromilow's model [27] was applied in further analysis to classify the analyzed compounds based on their potential to be systemic or contact pesticides. The crucial parameters (logP and pK_a) for Bromilow's model of each compound are presented in Table 3.

Table 3. In silico lipophilicity (consensus logP), pK_a and polarizability of the analyzed compounds.

Compound	Consensus logP	pK_a	Polarizability
Dicamba	2.53	2.5	18.632
Glyphosate	−2.30	−0.6	12.673
Atrazine	2.30	14.5	22.582
Simazine	1.95	14.8	20.751
1	2.83	14.5	24.990
2	2.63	14.3	24.425
3	3.57	14.6	28.191
4	3.49	14.2	28.669
5	3.70	14.1	30.397
6	4.53	14.1	34.316
7	5.36	14.1	38.147
8	6.28	14.1	41.117

Hydrophilic substances tend to behave as systemic pesticides, which depend on logP and pK_a. Their translocation can be done via phloem or xylem transport tissues in vascular plants. The analyzed triazines possess a basic character due to the basicity of nitrogen atoms in the triazine ring. Moreover, according to the pK_a values, the studied triazines (**1**–**8**) are very weak acids (the protons on ammine groups are not easily released). Based on Bromilow's diagram (Figure 4), it can be noticed that only compounds **6**, **7,** and **8** possess logP > 4 meaning that they are potential contact herbicides, while the rest of the compounds have logP < 4, classifying them as potential systemic pesticides among which the majority is transported by xylem and only glyphosate and dicamba can be transported by both transport systems xylem and phloem. In the diagram, compound **1** is very close to atrazine, so behavior similar to atrazine is expected. Compounds **3**, **4,** and **5** are close to the logP limit (the red vertical line that divides systemic and contact pesticides) but still in the zone of xylem mobility. Nevertheless, further investigations of these compounds are needed. Glyphosate and dicamba, as acidic non-triazine herbicides, are in the zone of phloem and xylem mobility.

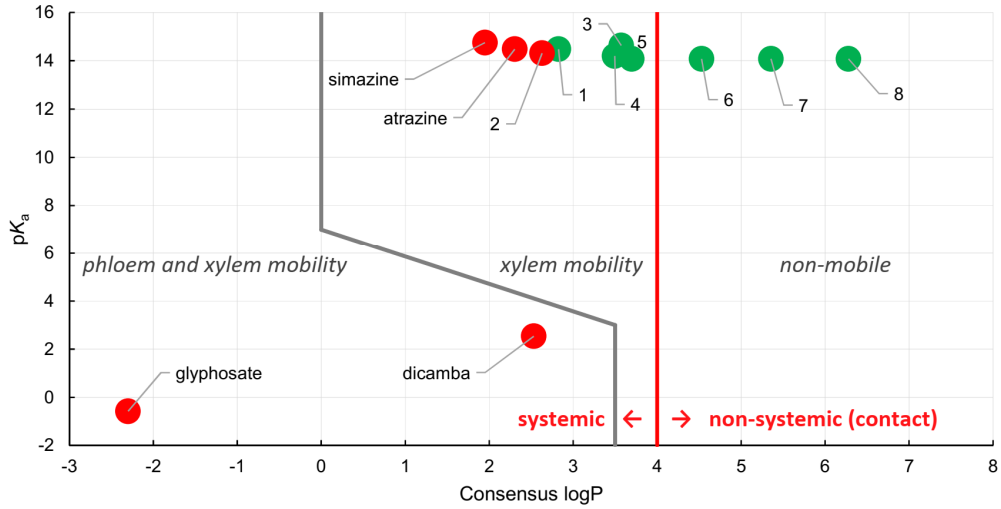

Figure 4. The classification of the studied compounds on Bromilow's diagram: the red dots mark the commercially available triazine (atrazine and simazine) and non-triazine herbicides (glyphosate and dicamba), including compound **2** (propazine) which belongs to the set of the evaluated triazines as a structural isomer of compound **1**; the green dots represent the potential herbicide candidates from the series of compounds **1**–**8**.

The conducted comparative analysis based on Bromilow's approach indicates that the analyzed triazine compounds (**1**, **3**–**5**) can be considered potential systemic herbicide candidates in view of their similarity regarding molecular structure, lipophilicity, and acidity. Considering the fact that simazine and propazine are absorbed via the root and translocated to the xylem, and that they have the longest action among the other triazine herbicides [6], the structural similarity of compounds **3** and **4** may indicate a similar mode of action. Compounds **6**–**8** can be potential contact herbicides of higher lipophilicity than commercial ones, so they could be used as pre-emergence herbicides or possibly early after the weeds and crops have sprouted from the soil.

If not well absorbed in a plant, compounds **6**, **7,** and **8** may be used as protective fungicides if they express fungicidal activity. On the other hand, well-absorbed and systematically translocated compounds may be used for curative treatment if they have fungicidal activity to inhibit the growth of fungal hyphae in leaves.

From the aspect of environmental contamination, compounds **1**–**4** have higher volatilization potential than compounds **5**–**8**, considering their polarizability (Table 3) and molecular weight as the key parameter for the assessment of this feature [28]. The *s*-triazines

with cycloalkyl substituents have a lower risk of air contamination than *s*-triazines with acyclic substituents.

3.2. Antifungal Activity towards Aspergillus flavus

The antifungal activity of the studied *s*-triazines was tested towards *A. flavus* PA2D SS strain, which is an opportunistic fungal pathogenic microorganism. It is very important since it has confirmed aflatoxigenic potential [31].

The results of the analysis of antifungal activity are presented in Figure 5. The antifungal activity is presented as inhibition zone diameter (IZD, mm) for each studied compound. The values of inhibition zone diameter are followed by standard deviation values. Based on the obtained inhibitory activity, it can be concluded that all the analyzed compounds have similar and significant activity; however, certain differences can be noticed.

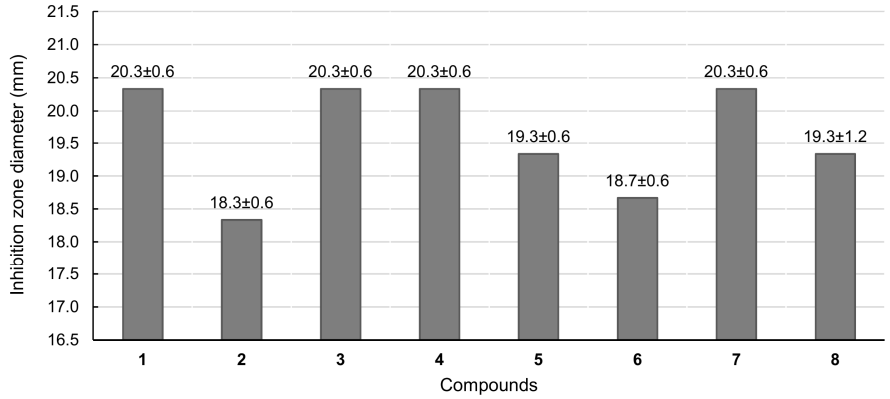

Figure 5. The results of microbiological analysis of fungicidal activity of the compounds **1**–**8** towards *A. flavus* PA2D SS strain (1 mg of triazine per well).

Namely, three out of four triazines with acyclic substituents (compounds **1, 3**, and **4**) and one out of four triazines with cyclic substituents (compound **7**) expressed the highest fungicidal activity (20.3 mm IZD). Compound **2** (propazine) expressed the lowest activity (18.3 mm IZD), followed by compounds **6** (18.7 mm IZD) and **5** (19.3 mm IZD).

There is not much available data regarding *s*-triazine antifungal activity towards *A. flavus*. Some novel nitro-1,2,4-triazine derivatives showed no inhibitory activity towards *A. flavus* [32], while some symmetrical and unsymmetrical triazine Schiff bases and their nickel (II), cobalt (II), zinc (II), and copper (II) complexes showed significant antifungal activity against *A. flavus* [33]. It is worth mentioning that some newly designed 1,2,4-triazine derivatives were tested on the *A. fumigatus* strain and expressed an inhibitory effect [34]. Considering compounds **1**–**8**, it can be said that they possess significant inhibitory potential towards *A. flavus*; however, strategies for improving their fungicide effect (e.g., complexation with metal ions) should be considered in further research.

3.3. Molecular Docking and Antifungal Activity: A Comparative Analysis

Molecular docking analysis was carried out on the enzymes of *A. flavus*. Compounds **1**–**8** were docked in the active sites of the enzymes, among which the oxidoreductases and transferases were the most represented. The docking results were based on the root mean square deviation (RMSD) lower bound (RMSD-l.b.) values. RMSD-l.b. indicates the conformational changes of a ligand in a binding site and represents the comparison of the atoms in one conformation with the closest atom of the same group in a different conformation. Moreover, the docking procedure is validated based on RMSD-l.b. values. If the RMSD-l.b. > 2 Å, the docking procedure must be repeated due to the huge differences between the structure of the docked ligand in its validated conformation (usually in crystal form) and the conformation predicted by the applied docking procedure [29].

The active site was detected based on the binding site of the corresponding ligand in the crystal structure of the enzyme. The best conformation of the triazines in the

active site was defined based on the lowest binding energy (kcal/mol). Then, other possible conformations of triazines in the active site were compared with the conformation described with the lowest binding energy by calculating the RMSD-l.b. values. The conformations described with RMSD-l.b. values greater than 2 Å were not taken into consideration. The docking procedure was validated by the docking of native ligands into the active site and measuring the RMSD values that were less than 2 Å.

Figure 6 shows the binding affinities of the studied triazines towards corresponding enzymes (lowest binding energy means highest binding affinity). The results indicate that triazines with acyclic substituents express the highest binding energies (lowest binding affinities) towards the majority of the enzymes. On the other hand, the triazines with cycloalkyl substituents have the lowest binding energies (highest binding affinities) towards the active sites of enzymes. This could imply that more lipophilic triazines have higher binding potential towards the active sites of the majority of the analyzed enzymes. In Figure 6, there is a clear separation of the analyzed compounds **1**–**8** into triazines that have acyclic substituents and triazines with cyclic substituents.

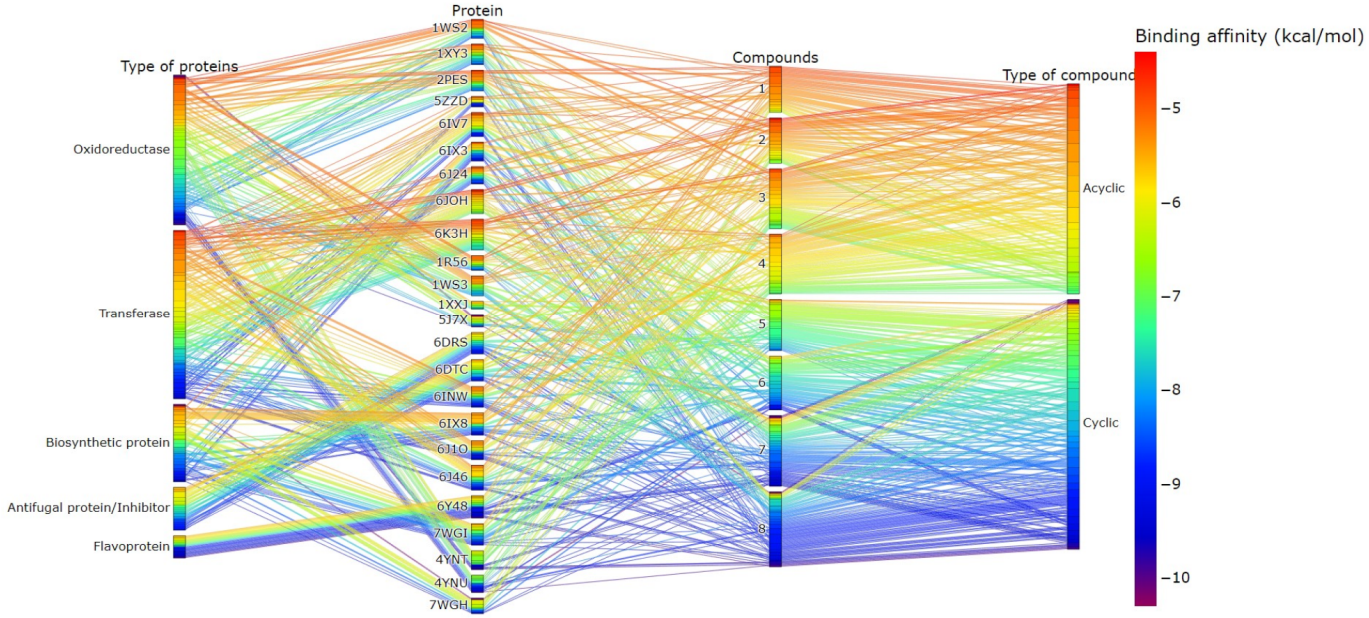

Figure 6. Binding affinity energies (kcal/mol) of the studied triazines (**1**–**8**) towards the enzymes of *A. flavus*.

The most common interactions between compounds **1**–**8** and the active sites are hydrogen and hydrophobic interactions. Hydrophobic interactions are more typical for triazines with cycloalkyl substituents, particularly cycloheptyl and cyclooctyl. Among the compounds **1**–**4** (with acyclic substituents), compound **4** has generally lower binding energies than the others. Compound **1** generally binds to the active sites of most of the considered enzymes at higher energies.

It should be emphasized that the interpretation of the results based on binding energy does not necessarily correlate with the results of cell culture assays [35]. Indeed, in the present study, the docking results interpreted via binding affinity do not correlate with determined fungicidal activity. Nevertheless, the binding affinities towards the analyzed enzymes of *A. flavus* are significant.

Figure 7 represents the validated interactions between compounds **1**–**8** and the active sites of the enzymes. Each dot represents one out of nine possible positions that are described by the RMSD-l.b. $\leq$ 2 Å [29]. The positions are ranked so the first one represents the lowest and the last one the highest binding energy. Most of the analyzed compounds have validated positions in the active sites of most of the corresponding enzymes. Compounds **7** and **8** have the validated dockings for almost all enzymes (23 out of 24).

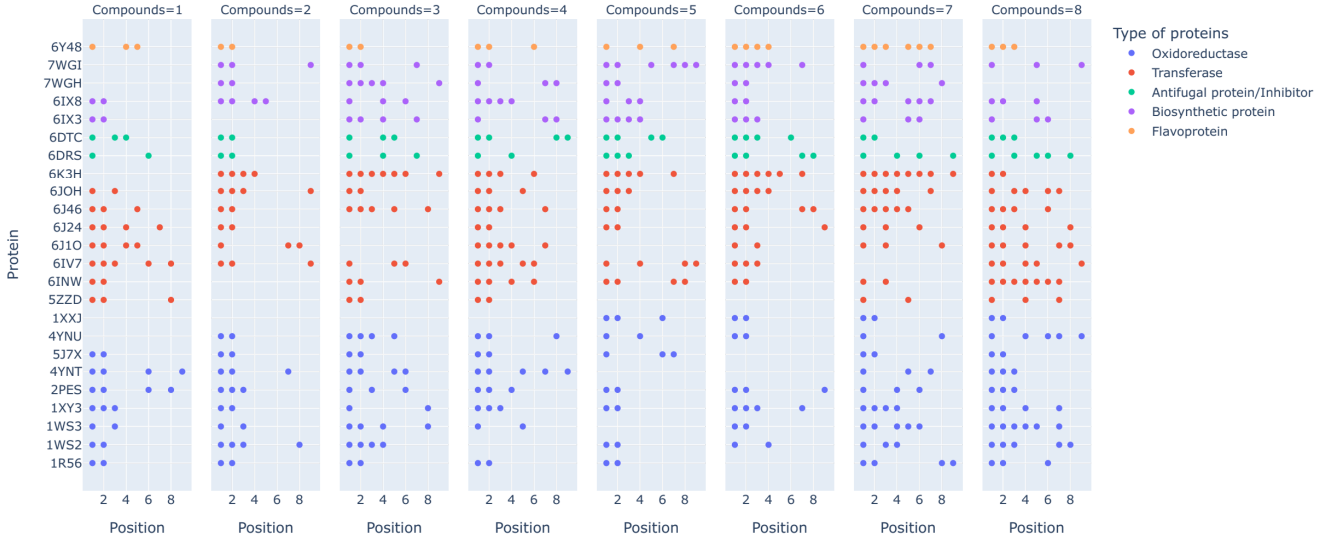

Figure 7. Validated binding positions (RMSD-l.b. $\leq$ 2 Å) of compounds **1**–**8** in the active sites of the corresponding enzymes.

Particularly interesting is the observation that all the compounds have validated docking positions in the active site of the enzyme 6IX8 (S-adenosylmethionine-dependent pericyclase), which was superimposed with the *Omt-1* protein [36], which plays a crucial role in the final steps of aflatoxins synthesis (AFB_1 and AFB_2) in *A. flavus* and *A. parasiticus* [37]. The docking of compounds **1**–**8** in the active site is presented in the Appendix (Figures A1 and A2). The highest affinities towards this enzyme may have the compounds **7** and **8** taking into account their lowest binding energies (Table A2). According to the data presented in Table A2, it can be seen that exactly these two compounds have the highest number of hydrophobic interactions. This type of interaction occurs in all of the analyzed triazines, while the H-bond is being formed with all the compounds with acyclic substituents (**1**–**4**) and two compounds with cycloalkyl substituents (**7** and **8**). Generally speaking, the conducted docking analysis indicates higher inhibition potential of cycloalkyl than acyclic derivatives towards biosynthetic protein 6IX8, and presumably *Omt-1* protein, taking into account the lowest binding energies and numerous hydrophobic bonds, as well as several H-bond and halogen-bond interactions.

3.4. Interactions of the s-Triazine Derivatives with Cereals Proteins

The molecular docking analysis of compounds **1**–**8** and commercial herbicides (atrazine, simazine, glyphosate, and dicamba) provided the binding energies and RMSD-l.b. values for each compound. The docking of native ligands into the active site was used for docking procedure validation. The RMSD values were under 2 Å; therefore, the conducted procedure can be considered valid.

The binding energies are presented in the form of a parallel graph in Figure 8. Generally, there can be seen that alkyl derivatives (**1**–**4**) bind with higher binding energies in most of the proteins than cycloalkyl derivatives (**5**–**8**). The commercial compounds, regardless of their molecular structure (triazine or non-triazine), also express lower binding energies towards the majority of the analyzed proteins than alkyl derivatives.

Having a higher binding affinity towards the cereal proteins, cycloalkyl derivatives (particularly compounds **7** and **8**) may have an inhibitory effect on crops, which can disqualify them as potential herbicides for the protection of *Hordeum vulgare* (barley), *Zea mays* (maize), *Oryza sativa* (rice), and *Triticum aestivum* (common wheat) crops.

In order to estimate the similarities and dissimilarities among the alkyl and cycloalkyl triazine derivatives and commercial herbicides (atrazine, simazine, dicamba, and glyphosate) regarding their binding affinity towards the enzyme of selected crops, and to si-

multaneously examine the grouping of enzymes according to the affinity of the investigated substances to them, a double dendrogram was formed applying HCA (Figure 9).

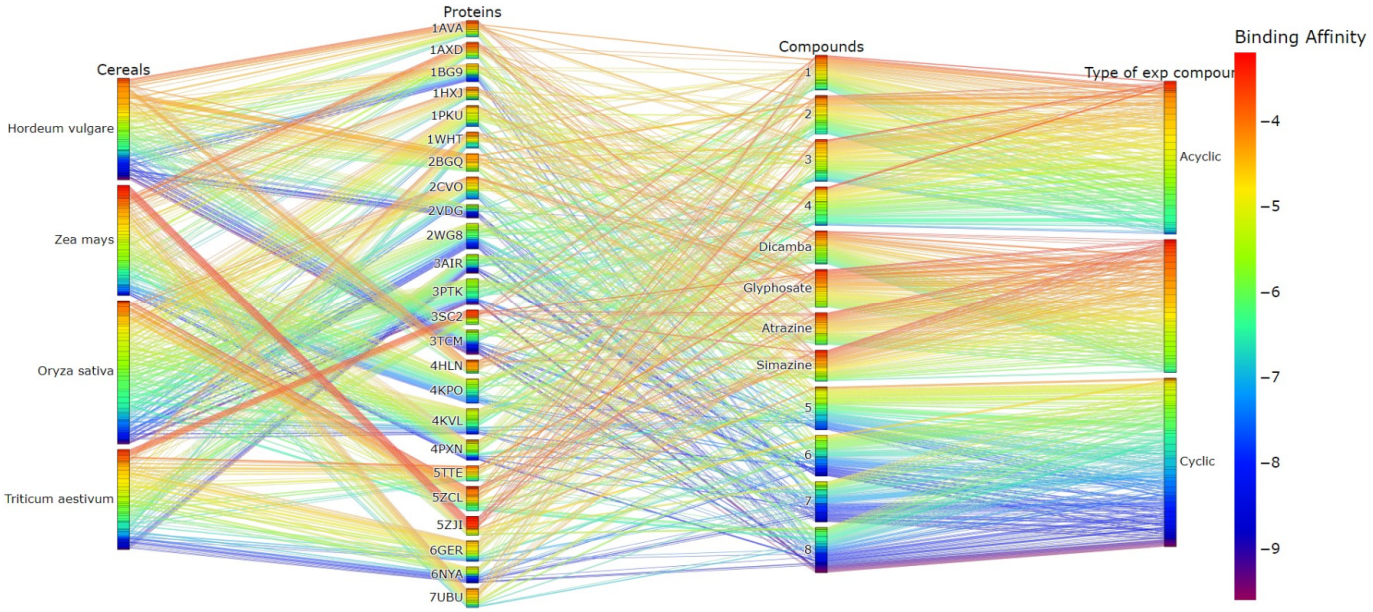

Figure 8. Binding affinity energies (kcal/mol) of the studied triazines (**1**–**8**) towards the enzymes of cereals *Hordeum vulgare* (barley), *Zea mays* (maize), *Oryza sativa* (rice), and *Triticum aestivum* (common wheat).

The vertical dendrogram indicates that compounds **6**, **7**, and **8** possess the highest affinity towards most of the enzymes compared to the rest of the compounds that were put in a separate cluster. In the second cluster, there are all the acyclic derivatives together with compound **5** and commercial herbicides. Therefore, despite the fact that compound **5** is a cycloalkyl derivative, it was placed together with alkyl derivatives in the space of the analyzed binding energies, implying their similarity in this instance. Moreover, the structural isomers (compounds **1** and **2**, as well as **3** and **4**) do not belong to the same cluster, suggesting the significant influence of structural isomerism on the binding affinity in some enzymes.

Atrazine, as one of the commercial triazines, is widely used as a selective herbicide in maize fields thanks to the fact that maize has biochemical resistance to atrazine [6]. Based on the presented dendrogram, it can be seen that atrazine has quite a low affinity towards maize transferases; besides, there were no validated docking positions of atrazine in maize oxidoreductases (5ZJI and 4PXN) and a hydrolase (4KPO), suggesting maize resistance to atrazine (which was confirmed earlier [6]). Among compounds **1**–**8**, compound **7** has no validated docking position in some hydrolases (1HXJ from maize, 3AIR from common wheat, and 3PTK from rice) and transferases (4HLN from barley, 1PKU from rice), which makes it particularly interesting for further studies of the potential biochemical resistance of the cereals towards the cycloheptyl−triazine derivative. Generally speaking, cycloalkyl derivatives are more likely to be unsuitable herbicides for application in common wheat, barley, rice, and maize crops, due to their high binding affinity towards the majority of the enzymes of the cereals taken into account.

The horizontal dendrogram does not show the grouping of the cereals or the enzymes regarding their functions and types. This indicates the non-selective action of the compounds towards selected cereals and protein groups.

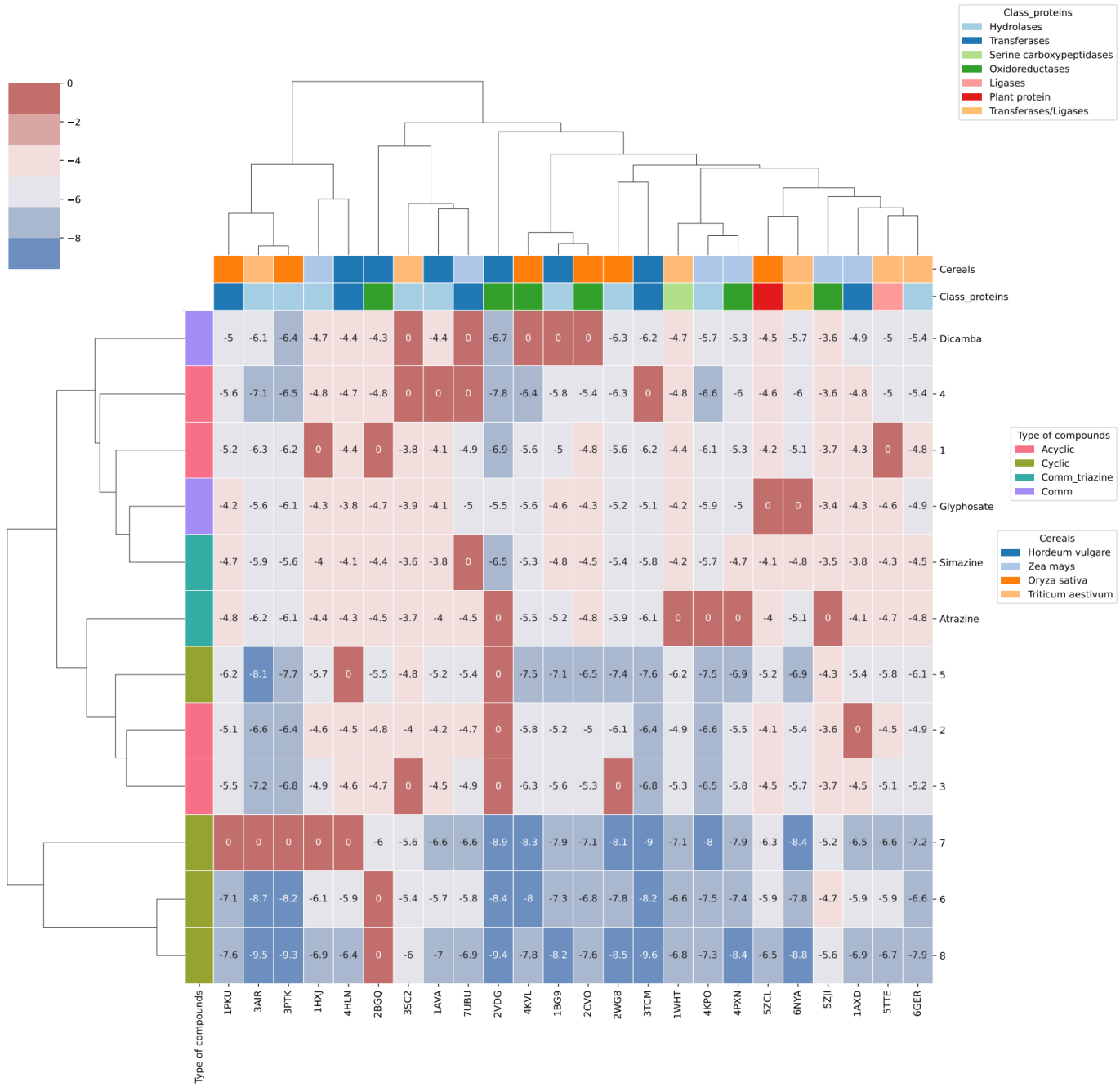

Figure 9. Hierarchical clustering of the studied triazines (**1**–**8**) and the proteins of considered cereals based on binding energies (acyclic—compounds **1**–**4**; cyclic—compounds **5**–**8**; Comm—dicamba, glyphosate; Comm_triazine—atrazine, simazine).

4. Conclusions

Based on the presented results, it can be concluded that the studied alkyl and cycloalkyl derivatives have lipophilicity parameters in the range, so they fall into the group of xylem mobile (compounds **1**–**5**) and non-mobile or contact herbicides (compounds **6**–**8**). Compounds **6**–**8** can be considered potential contact pre-emergence herbicides due to their higher lipophilicity than commercial ones. Alkyl derivatives pose a higher risk of air contamination than cycloalkyl derivatives due to their higher polarizability. All the analyzed compounds expressed significant antifungal activity towards *A. flavus*, among which compounds **1**, **3**, **4**, and **7** possess the highest activity. The docking analysis also indicated the higher inhibition potential of cycloalkyl than alkyl derivatives towards biosynthetic protein

6IX8, which was superimposed with the *Omt-1* protein, implying that cycloalkyl derivatives have a higher potential of inhibition of aflatoxins synthesis in *A. flavus* and *A. parasiticus*. The molecular docking analysis also led to the conclusion that cycloalkyl derivatives are more likely to be unsuitable herbicides for application in common wheat, barley, rice, and maize crops. Hierarchical clustering revealed no grouping of the cereals or enzymes regarding their functions and types, so the non-selective action of the compounds towards selected cereals and protein groups was observed. Despite the fact that compounds **1** and **2**, as well as **3** and **4**, are structural isomers, they have significantly different binding affinities towards some cereal proteins. The comparative analysis showed significant similarities between the acyclic triazines and some commercial triazines (atrazine and simazine) and non-triazine herbicides (dicamba and glyphosate) in terms of their binding affinity towards the majority of the analyzed enzymes.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Lipophilicity descriptors of some commercial triazine and non-triazine herbicides.

Compound	ALOGPS 2.1 Program						SWISS$_{ADME}$		MarvinSketch			ChemDraw		Consensus logP
	ALOGPs	AClogP	AlogP	MlogP	XlogP2	XlogP3	iLOGP	WLOGP	logPVG	logPKLOP	logPPHYS	LogPChDr	CLogP	
atrazine	2.70	2.48	2.54	2.59	1.66	2.61	2.73	1.40	2.06	2.40	2.13	1.95	2.70	2.30
simazine	2.48	2.08	2.16	2.27	1.20	2.18	2.60	1.01	1.65	1.97	1.73	1.63	2.39	1.95
dicamba	2.65	2.62	2.75	2.62	2.80	2.21	1.70	2.70	2.35	2.97	2.73	2.58	2.24	2.53
glyphosate	−2.43	−4.81	−2.07	−1.96	−2.68	−4.62	−0.58	−1.20	−1.92	−1.92	−1.59	−0.45	−3.69	−2.30

Table A2. The binding energy and intermolecular interactions between the triazines **1**–**8** and the amino acids in the active site of enzyme 6IX8.

Compound	Binding Energy (kcal/mol)	Hydrogen Bonds	Hydrophobic Interactions	Halogen Bonds
1	−5.5	ASP-195, ARG-197, GLY-228, ARG-291 (2) *	ARG-291 LEU-192, LEU-193,	-
2	−5.5	ARG-291 (2)	ARG-197, LEU-292, ILE-293, LEU-193, GLU-196	ASP-225
3	−5.8	ILE-186	PHE-176, LEU-179 (2), ILE-186, PHE-189 (2), LYS-190 (2), LEU-193, VAL-256, ALA-259	-

Table A2. *Cont.*

Compound	Binding Energy (kcal/mol)	Hydrogen Bonds	Hydrophobic Interactions	Halogen Bonds
4	−5.6	GLY-227, HIS-232, ARG-291 (2)	ARG-197, LEU-193, LEU-292	ASP-225
5	−7.3	-	PHE-176, LEU-179, PHE-189, LEU-253 (3)	ILE-293
6	−8.1	-	LEU-179, ILE-186, PHE-189, LEU-253 (3)	-
7	−8.7	ASP-252	LEU-179 (2), ILE-186, PHE-189 (4), LEU-253 (3), PHE-276 (4)	LEU-292
8	−9.4	ASP-252	LEU-179 (2), ILE-186 (2), PHE-189 (3), LEU-253 (2), PHE-276 (4)	-

* The numbers in brackets represent the number of the specific bonds established between the specific compound and the corresponding amino acid in the active site of enzyme.

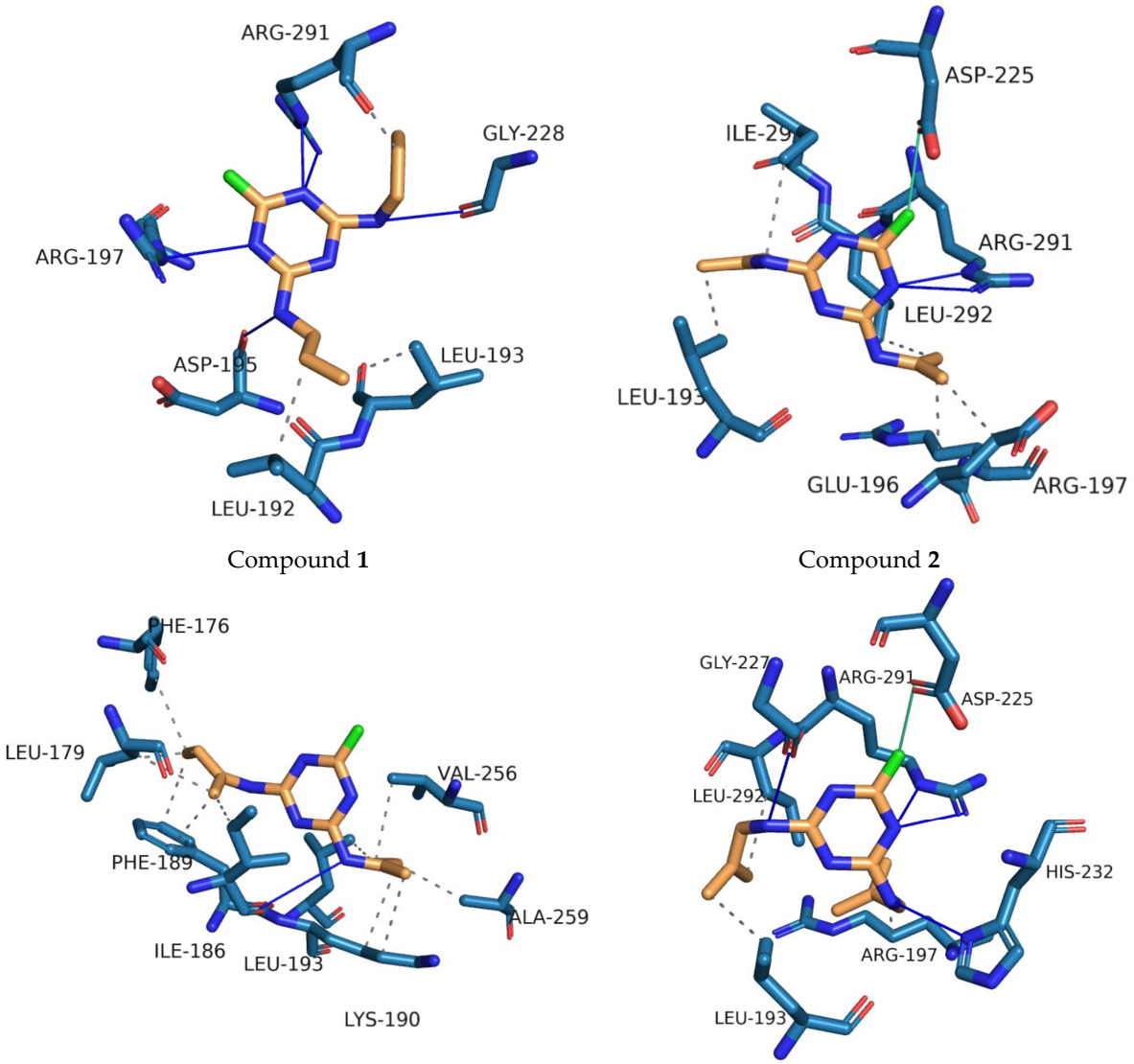

Figure A1. The lowest-energy positions of compounds **1**–**4** in the active site of 6IX8 enzyme.

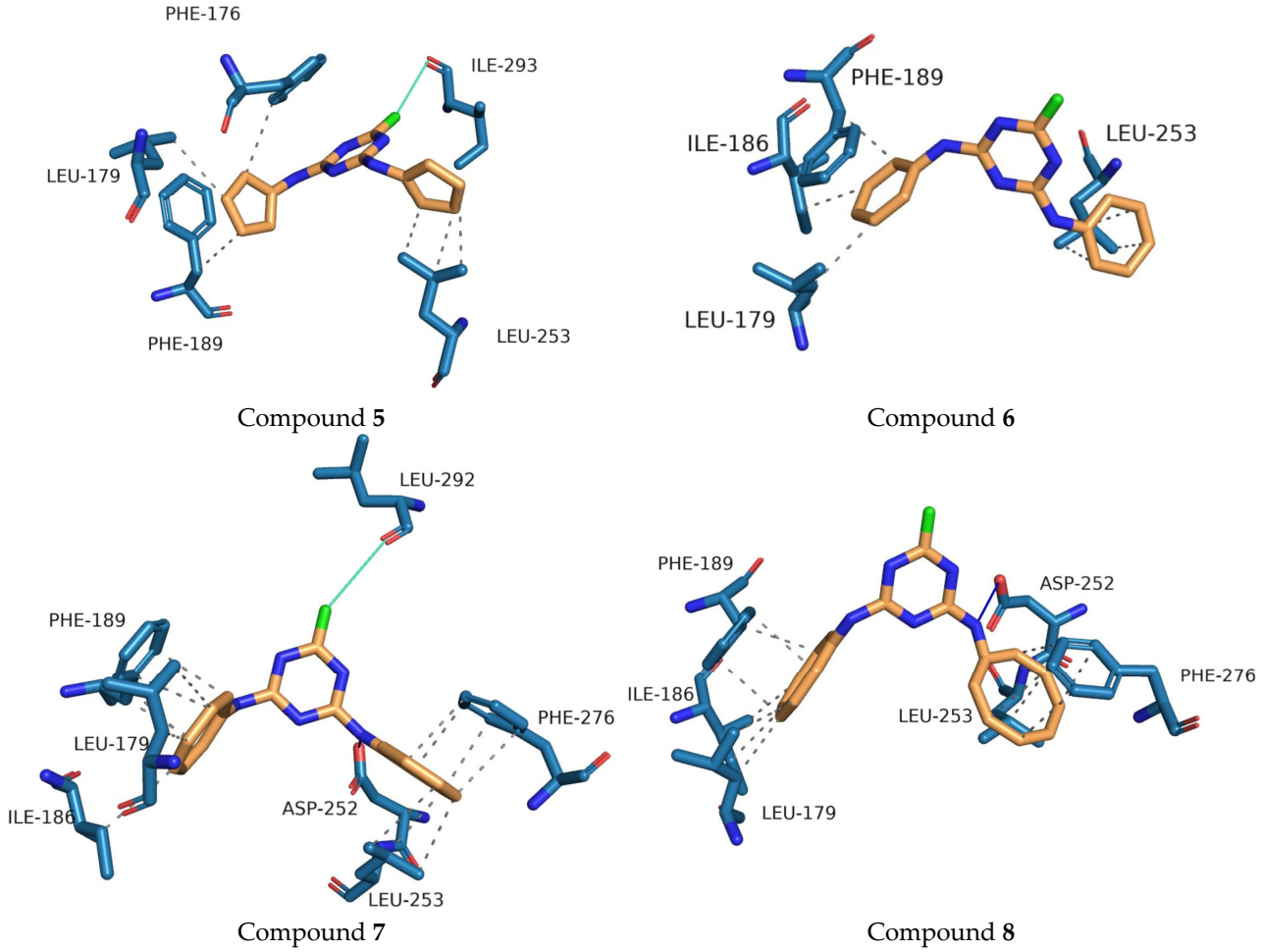

Figure A2. The lowest-energy positions of compounds **5**–**8** in the active site of 6IX8 enzyme.

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