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Prediction of selected properties of aflatoxin molecules by the QSAR method

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Abstract: Aflatoxins are naturally occurring compounds produced by fungi, mainly of *Aspergillus* species. All aflatoxins are proved to cause acute toxicity to human health, some even causing chronic diseases such as cancer. These molecules clearly proved that even natural molecules can be directly related to cancer and the formation of tumours. In general, aflatoxins can be characterised as organic compounds; B1, G1, B2, and G2 are produced directly by fungi and these four are subsequently metabolised in biological systems of microbes, animals or humans into other forms, such as M1, EB1, AFL etc. This manuscript provides a brief overview of 14 aflatoxins, their molecular structure and its possible relationship to aflatoxins biological activity. This information, in combination with additional calculations, offers the possibility to investigate the mentioned compounds and their properties using the QSAR approach**.**

Keywords: aflatoxins, biological activity, QSAR analysis, principal component analysis

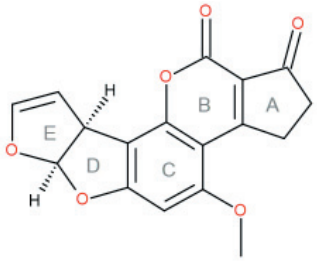
Introduction

Aflatoxins, mycotoxins produced exclusively by fungi, have been studied extensively since the 1960's. These compounds have proved that molecules of purely natural origin can cause not only acute toxicity, but also chronic diseases including immune suppression and malignant cell growth (Hsieh, 1988; Pickova et al., 2021). Liver and kidney cancer diseases are typically associated with aflatoxins and their intermediate metabolites (Benkerroum, 2020a). However, the risk to human body is via the ingestion of contaminated food crops, as well as the consumption of milk, eggs, meat and products made of them in case of livestock and poultry intoxication during feeding. Intensive monitoring of these substances showed their frequent occurrence in agricultural crops. Public concern about safety and quality crop products has increased extensively, forcing regulatory authorities and related stakeholders to develop robust safety evaluation and control policies. (Bennett and Klich, 2003; Elgioushy et al., 2020; World Health Organization, 2018; European Commission, 2006a; European Commission, 2006b).

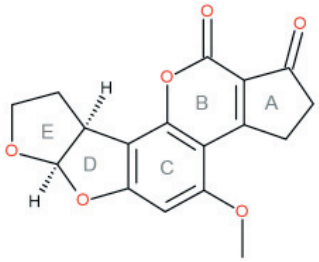
Chemical structure of a molecule has a direct impact on its properties such as bioactivity at the cellular level. Increased probability of toxicity is observed for less polar and more lipophilic compounds (Hughes et al., 2008). Molecular lipophilicity can be expressed as the base ten logarithm of the octanol-water partition coefficient (Log P); lipophilic substances are more soluble in octanol than in water and the value of this coefficient is above zero. On the other hand, negative Log P values indicate hydrophilic substances. The Log P quantity can also be evaluated theoretically using the density functional theory combined with an implicit solvent model (Michalík and Lukeš, 2016). Calculated Log P values correlate well with biological concentration of phenols and their possible quinone metabolites (Michalík et al., 2018a), 3-hydroxynaphthalene-2-carboxanilides (Michalík et al., 2018b), 1-hydroxynaphthalene-2-carboxanilides, and 2-hydroxynaphthalene-1-carboxanilides (Škorňa et al., 2017). Empirical Log P values can be determined using the approaches of chemical informatics, namely the Quantitative Structure-Activity Relationship (QSAR) approach (Muratov et al., 2020). Generally, the QSAR method allows generating relationships between structures and selected chemical and biological properties of molecules.

Based on the severity of the effects aflatoxins have on human health, there are publications devoted to systematic analysis of their chemical structure and its relationship to their biological activity or reactivity (Goldblatt, 1969; Heathcote and Hibbert, 1978; Betina, 1990). Most of available studies are found in the field of toxicology and agricultural sciences, where experimentally selected properties regarding aflatoxins mutagenic or carcinogenic potential are observed for only a few molecules (Wong and Hsieh, 1976). It is also possible to find various studies in the field of chemistry, but these mostly analyse the reactivity of the molecules (Valerio et al., 2007). Regarding experimental data, information on relative mutagenicity is available through the Ames test (Tejs, 2008), which evaluation includes the number of revertants, i.e. the number of *Salmonella* bacteria cells that restored a mutation in their histidine operon. A systematic view of the chemical structure and an

estimate of its possible influence on properties influencing biological effectiveness are missing. For this reason, the study of aflatoxin molecules at the theoretical level was chosen.



Aflatoxin B1 (**B1**)



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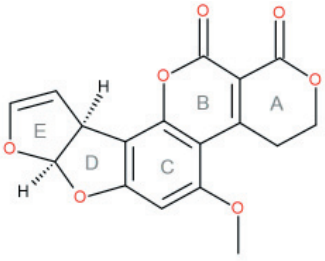
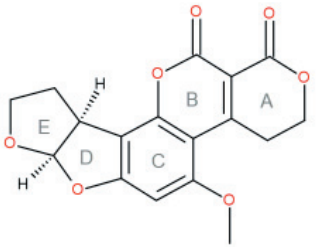
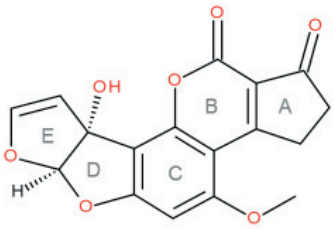


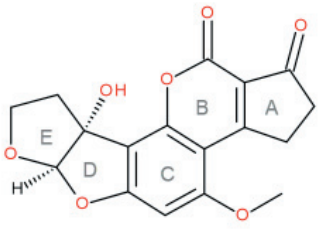
Aflatoxin G1 (**G1**)



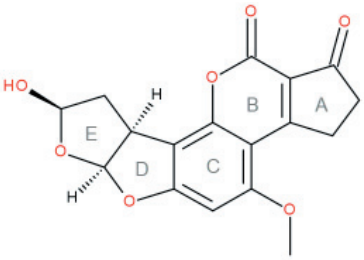
Aflatoxin G2 (**G2**)



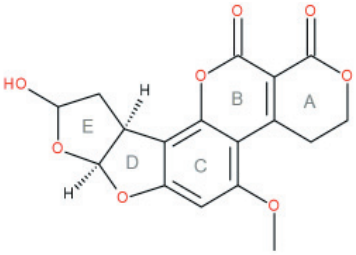
Aflatoxin M1 (**M1**)



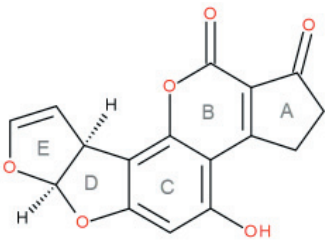
Aflatoxin M2 (**M2**)



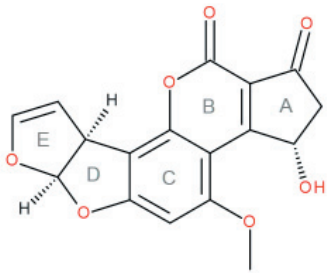
Aflatoxin B2a (**B2a**)



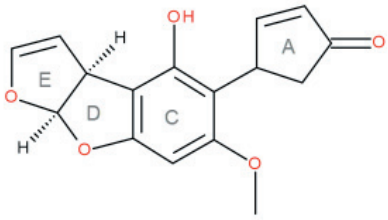
Aflatoxin G2a (**G2a**)



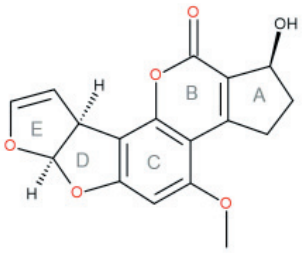
Aflatoxin P1 (**P1**)



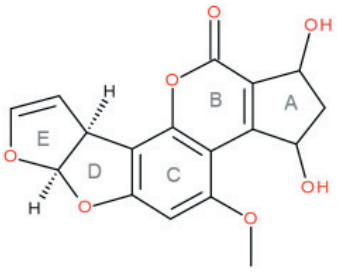
Aflatoxin Q1 (**Q1**)



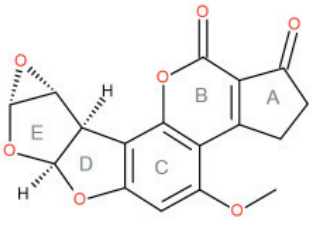
Aflatoxin D1 (**D1**)



Aflatoxicol (**AFL**)



Aflatoxicol H1 (**H1**)



2,3-epoxyaflatoxin B1 (**EB1**)

Fig. 1. Structural formulas and ring notations of studied molecules.

The main aims of the study are: (1) discuss the chemical structure of selected aflatoxins (see Fig. 1), (2) determine chemical and biological properties of the studied molecules using the QSAR method, (3) correlate calculated parameters using statistical principal component analysis (PCA), and (4) determine possible corelation between the evaluated QSAR values with available experimental data for relative mutagenicity of the studied molecules.

Methodology

QSAR is a method based on known experimental data and a statistical model, which enables to determine mathematical quantitative relationship between the structure and activity of the molecule. Properties such as topological polar surface area (TPSA), lipophilicity (miLogP), G-protein-coupled receptors (GPCR), kinase inhibitor (KI), nuclear receptor ligand (NRL), enzyme inhibitor (EI), and protease inhibitor (PI) were monitored. Molinspiration supports internet chemistry community by offering free online services for calculation of the above mentioned important molecular properties (Molinspiration Cheminformatics, 2023). The TPSA descriptor expresses the sum of polar atoms surface area of a molecule; this relevantly characterises the transport of a molecule including its absorption and bioavailability (Kulkarni et al., 2021). According to the Veber's rule (Veber et al., 2002), molecules with TPSA value of 140 $Å^2$ or lower are likely to have good intestinal absorption (Viswanadhan et al., 1989). The miLogP coefficient represents lipophilicity, indicating the degree of molecular hydrophobicity, which directly affects the ability of the molecule to penetrate the cell membrane, its metabolism, and quantifies the hydrophobic interactions at the molecule-receptor level (Soliman et al., 2021).

GPCR, KI, NRL, EI and PI parameters are used to predict biological activity. Biological activity score for organic molecules can be interpreted as active when its value is higher than 0, moderately active when it is between –5.0 and 0.0, and inactive when it is lower than –5.0 (Flores-Holguín et al., 2019).

GPCR parameter describes the biological activity of individual molecules in terms of whether the given molecule can bind to G-protein-coupled receptors. The receptor thus activates intermediate products called G-proteins (Srivastava, 2021), which subsequently activate enzymes, open ion channels, and initiate intracellular signalizing cascades.

Kinase inhibitor is a substance that blocks a type of enzyme called kinase. This feature is characterised by the KI parameter. Human cells have many different kinases which help control important functions such as cell signalizing involved in many diseases including cancer, their metabolism, division, and survival (Kulkarni et al., 2021).

Ligands that show the ability to bind to nuclear receptors and thereby activate them are called ligands for nuclear receptors (NRL). Typically, ligand-biomolecule complexes are formed based on non-covalent interactions (Burdge and Overby, 2020). They include lipophilic substances such as endogenous hormones, vitamins A and D, and xenobiotic hormones. Since the expression of a large number of genes is regulated by nuclear receptors, ligands that activate these receptors can have a significant effect on the organism (Sharma and Raghav, 2021).

Protease inhibitors (PI) are compounds that act by interfering with enzymes that cleave proteins, e.g. inhibitors preventing viral replication by selectively binding to viral proteases and blocking proteolytic cleavage of protein precursors (Shamsi et al., 2021).

Enzyme inhibitors (EI) are substances that block the action of an enzyme. Enzymes help speed up chemical reactions in the body and are involved in many cell functions, including cell signalizing, growth, and division. In cancer treatment, enzyme inhibitors can be used to block certain enzymes that cancer cells need to grow (Kulkarni et al., 2021).

Principal Component Analysis (PCA) can be used for larger sets of data, where possible intercorrelations are expected. This statistical approach is based on orthogonal data transformation allowing to create new element sets. By visualizing them, correlations between groups of evaluated properties can be identified (Sheoran et al., 2020). This statistical approach can show the existence or non-existence of a correlation between the selected properties.

Results

The subject of research are 14 selected aflatoxins. Molecules of aflatoxin **B1** and **G1**, together with their hydrogenated derivates **B2** and **G2** are those best described in literature. Furthermore, there are aflatoxins **M1** and **M2**, which are the 4-hydroxy and 4-dihydroxy derivates of **B1** and **B2,** respectively. Other investigated molecules are their known derivates or metabolites of animal metabolism. An overview of investigated molecules is provided in Figure 1.

From the point of view of organic chemistry, chemical structure of the studied molecules can be limited to three basic fragments formed by five-membered or six-membered rings. The first fragment is formed by only one cyclic ring A, which ensures the greatest variability in structures. In aflatoxins **B1**, **B2**, **B2a**, **M1**, **M2**, **P1**, **Q1**, **AFL** and **H1**,

ring A represents cyclopentene. For aflatoxins **G1**, **G2**, **G2a**, cycle A is a 6-membered lactone ring. The second fragment represents coumarin containing rings B and C, where ring C may contain a methoxy or hydroxyl group. Analogous to hydroxyl group, the methoxy group can also represent important reaction site in metabolic processes and chemical reactivity. For example, the position of methoxy groups has direct influence on anti-cancer chemopreventive effects as inhibitors of P-glycoprotein (Štellerová et al., 2022). In case of guaiacol oxidation, methoxy group demethylation significantly affects thermochemistry of homolytic abstraction of the hydrogen atom from the neighbouring hydroxyl group (Biela et al., 2022). This abstraction leads to cyclization or quinone molecule formation. The hydroxyl group itself represents a potential site of metal ion chelation (Michalík et al., 2021). The third fragment consists of two difuran nuclei D and E, where a double or single bond can alternate on ring E. At the same time, one hydroxyl group can appear on this fragment. In case of the connecting bond between rings E and D, there are two alternative options for the orientation of the hydrogens, resp. hydrogen and a substituent. In this work, only one type of isomer was studied, where the hydrogen atoms or the hydrogen-substituent pair are oriented in the same direction. In this case, the third fragment is the least deformed and therefore more energetically advantageous. Based on this analysis, the selected group of aflatoxins can be divided into two main groups. The first group represents difurocumarocyclopentenones, which typically consist of a series of aflatoxins **B** and their derivates. The second group represents difurocumarolactones with the main representatives of the aflatoxin **G** series, including **G1**, **G2**, and **G2a**. It can be noted that aflatoxins with a saturated (**G2** and **M2**) or hydrated (**B2a**, **G2a**) terminal furan ring show the lowest toxicity. The most widespread and most dangerous aflatoxin **B1** has a special double bond in the cyclic ring E, which is also observed in aflatoxin **G1** and **M1**. Within this framework, several experiments, both *in vivo* and *in vitro,* showed that the potency of aflatoxin toxicity decreases in order **B1** > **G1** > > **B2** > **G2** (Zuckerman et al., 1967; Wogan et al., 1971, Veselý et al., 1983). This suggests the key role of the double bond in this furan group (Benkerroum, 2020b). Structures with a double bond on ring E have poor solubility in water and are prone to undergo an epoxidation reaction. In this regard, one of the direct metabolites of aflatoxin **B1**, molecule **EB1**, is considered as the principle responsible for genotoxicity. As an example of such **EB**1 action, its active isomer, *exo*-8,9-epoxide, interacts with DNA through S_N2 reaction mechanism creating a covalent bond with its guanyl N^7 atom (Johnson and Guengerich, 1997). Molecule **D1** is structurally different as ring B is not formed.

As for the QSAR analysis, based on known experimental data and a statistical model, a mathematical quantitative relationship between the structure and activity of a molecule can be determined. Table 1 summarises, in addition to the parameters mentioned above, basic properties such as molar mass (M), molecular volume (V), and number of atoms (N_{at}), given without hydrogen atoms.

The smallest volume is shown by molecules **P1**, **D1** and **B1**, and on the contrary, molecules **G2a** and **G**2 have the largest volume. All G-molecules contain a 6-membered ring (ring A), which causes a considerably larger bulk compared to the others. The highest value of TPSA is shown by aflatoxin **G2a**, which is closely related to the number of atoms and molar mass, as these values are the highest in **G2a**. It follows that the molecule with the lowest number of atoms and the smallest molar mass shows the lowest TPSA value. Specifically, it is a **D1** molecule that does not contain a B ring. As for miLogP, the highest value is recorded in case of **EB1**, which means that it has the highest ability to penetrate the cell membrane. On the other hand, compound **H1** showed the lowest ability to penetrate the cell membrane. By comparing the GPCR values, it can be concluded that all molecules show moderate biological activity in this property. The exception is the **D1** molecule, which is biologically active. Based on the values listed in Tab. 1, it can be concluded that all aflatoxins investigated show moderate biological activity and thus may have moderate effect on blocking kinases. By monitoring the NRL parameter, it was found that **B1** shows a significantly different value from the rest of the molecules as this value is negative, while for others it is positive. This means that **B1** has lower ability to bind to nuclear receptors than other molecules. Furthermore, it is evident that the PI values are negative and similar for all aflatoxins, therefore, all of them have moderate effect on blocking protein cleavage. In case of the EI parameter, **B1** and **G1** show only negative values, thus showing moderate biological activity. Other aflatoxins are biologically active and are therefore able to block the action of enzymes. It should be noted that this prediction is based on the molecular structure which affects the spatial conditions of chemical reactions. The common structural feature of compounds **B1** and **G1** is that they have a methoxy group on ring C, one double bond on ring E and no hydroxyl group is present. Nevertheless, the enzymatic reactions are a more complex problem as they are affected

also by thermodynamical processes and chemical kinetics. Unfortunately, to the best our knowledge, no experimental values are available in literature, therefore, correct estimation of molecular structure influence on the evaluated parameters is not possible.

Table 1 also shows experimental data for relative mutagenicity and the amount of revertants for the studied compounds (Wong and Hsieh, 1976). Mutagenicity represents a very complex quantity which is dependent on many factors end molecular properties. To better understand the generated QSAR data, statistical principal component analysis (Jolliffe and Cadima, 2016) has been done by linear transformation of the original data into a new coordinate system where the variation in the data can be described within a lower number of dimensions. The linear reliance between each pair of descriptors, Pearson's *r* correlation coefficients, is shown in Table 2. The best positively correlated descriptors are GPCR/KI with the magnitude of 0.852 and EI/PI with the magnitude of 0.796. Relationship between particular inhibitor activities may be related to similarities in QSAR design of enzyme receptor active sides as well as in relation of aflatoxin structures. As for the best negative correlation, TPSA/miLogP surface descriptors showed the value of –0.676. This relationship comes from the structural definition of the descriptors, where miLogP represents lipophilicity of studied aflatoxins and TPSA the proportion of polar groups in their structures.

In Figure 2, the correlation biplot shows the relationship between every QSAR parameter. Each feature is plotted as a vector with origin at point [0; 0]. Important information is the angle of the vectors.

Tab. 1. Properties generated by the QSAR approach (N_{at} is the number of atoms, *M* is molar mass, *V* is volume of the molecule, TPSA is surface area of the molecule, miLogP is lipophilicity, GPCR is a ligand for receptors coupled to G-proteins, KI is kinase inhibitor, NRL is a ligand for nuclear receptors and EI is enzyme inhibitor, PI is protease inhibitor). Experimental relative mutagenicity (RM) and the number of revertants (Rev) have been taken from ref. Wong and Hsieh, 1976.

Molecule	N_{at}	*M* g·mol^{-1}	*V* Å^3	TPSA Å^2	miLogP	GPCR	KI	NRL	EI	PI	RM	Rev
B1	23	312.28	253.24	74.98	1.48	–0.26	–0.43	–0.09	–0.09	–0.36	100	8527
G1	24	328.28	262.22	84.22	1.52	–0.24	–0.38	0.01	–0.01	–0.38	3.3	285
M1	24	328.28	260.93	95.21	0.90	–0.16	–0.42	0.21	0.18	–0.29	3.2	275
EB1	24	324.29	259.20	76.92	1.96	–0.25	–0.57	0.04	0.01	–0.40	–	–
B2a	24	330.29	267.47	95.21	0.91	–0.14	–0.41	0.15	0.25	–0.18	0.0	2
Q1	24	328.28	261.28	95.21	0.49	–0.07	–0.34	0.21	0.17	–0.22	1.1	99
P1	22	298.25	235.71	85.98	1.20	–0.23	–0.41	0.23	0.22	–0.33	0.1	10
G2a	25	346.29	276.45	104.45	0.95	–0.13	–0.36	0.07	0.15	–0.21	0.0	–
D1	21	286.28	244.13	65.00	1.25	0.23	–0.15	0.16	0.39	–0.14	–	–
B2	23	314.29	259.42	74.98	1.57	–0.22	–0.53	0.10	0.23	–0.19	0.2	18
G2	24	330.29	268.41	84.22	1.61	–0.19	–0.47	0.03	0.14	–0.20	0.1	9
M2	24	330.29	267.12	95.21	1.00	–0.13	–0.47	0.26	0.29	–0.16	–	–
AFL	23	314.29	259.10	78.14	1.14	–0.02	–0.28	0.34	0.20	–0.27	22.8	1940
H1	24	330.29	267.14	98.37	0.15	–0.05	–0.26	0.22	0.14	–0.20	2.0	170

Tab. 2. Pearson's *r* correlation coefficients for each pair of QSAR descriptors

Pearson's r	V	TPSA	miLogP	GPCR	KI	NRL	EI	PI
V	–	0.627	–0.244	–0.147	–0.191	–0.110	–0.154	0.290
TPSA	0.627	–	–0.676	–0.180	–0.045	0.262	0.022	0.210
miLogP	–0.244	–0.676	–	–0.409	–0.550	–0.580	–0.327	–0.469
GPCR	–0.147	–0.180	–0.409	–	0.852	0.483	0.679	0.621
KI	–0.191	–0.045	–0.550	0.852	–	0.389	0.392	0.367
NRL	–0.110	0.262	–0.580	0.483	0.389	–	0.683	0.400
EI	–0.154	0.022	–0.327	0.679	0.392	0.683	–	0.796
PI	0.290	0.210	–0.469	0.621	0.367	0.400	0.796	–

The cosine of the angle between a pair of vectors quantifies the correlation between the corresponding variables, cos 90 ° = 0, cos 180 ° = –1, cos 0 ° = 1. Therefore, vectors of uncorrelated properties are perpendicular to each other. The smaller this angle, the greater the correlation they describe. The sign of the correlation is then plus. When both angles are greater than 90 degrees, the correlation is also greater and the sign of the correlation is minus (Kohler and Luniak, 2005).

As illustrated in Figure 2, the vector of TPSA forms an almost right angle with the GPCR vector and a slightly smaller angle with the KI and EI vectors, which indicates the smallest correlation with TPSA among the selected properties. A greater correlation can be seen between the miLogP and NRL, PI vectors which make an angle of approximately 180 degrees, so the cosine of the angle is –1 and the correlation coefficients are negative. There is a fairly strong dependency between TPSA, *V* and GPCR, KI, EI vectors. The correlation coefficients are positive in both cases. Points that are close to each other represent objects with similar values of analysed properties. Points located in the lower part of the plot show similar behaviour for miLogP, GPCR, KI and EI properties; these are points **AFL**, **P1** and **B2**. Points **Q1**, **M2** and **B2a** are the closest to each other, and thus there is the greatest similarity between them. The same trend is shown for point **B1** and its metabolite **EB1**, which are placed distantly from the other points. An object at a large distance from the origin has a large interaction effect with at least one variable, aflatoxin **B1** and 2,3-epoxyaflatoxin **EB1** show this effect with miLogP. Point **G2a** is located approximately on the extended *V* vector due to the largest volume of its molecule among the monitored molecules. Points for compounds **H1** and **D1** stand alone, with no company of other molecules around; thus different behaviour of these molecules can be assumed. It is important to note that the lengths of the vector arrows indicate the characters with the greatest influence on the layout of the graph. However, most vectors are of similar length. It follows that these vectors have a similar effect, except for KI and *V*, with significantly shorter vectors. Thus, these QSAR descriptors have the lowest effect on the arrangement of the graph (Fig. 2).

In case of published experimental data, only four molecules can be selected with the best ratio between the measured value and the estimated measurement error. These include molecules **B1**, **AFL**, **Q1** and **H1**, where there is a double bond enabling the formation of an epoxy bond on the E ring. According to Wong and Hsieh (1976), B1 molecule exhibits the highest mutagenicity (Tab. 1). For **Q1** and **H1** molecules, possible conversion of hydroxyl groups into keto groups can play a specific role as the mechanism of such transformation is connected with proton and electron transfer processes (Lukeš et al., 2022). Despite the relative mutagenicity being mainly connected to aflatoxin epoxy metabolites; we tried to find a correlation between QSAR descriptors and available experi-

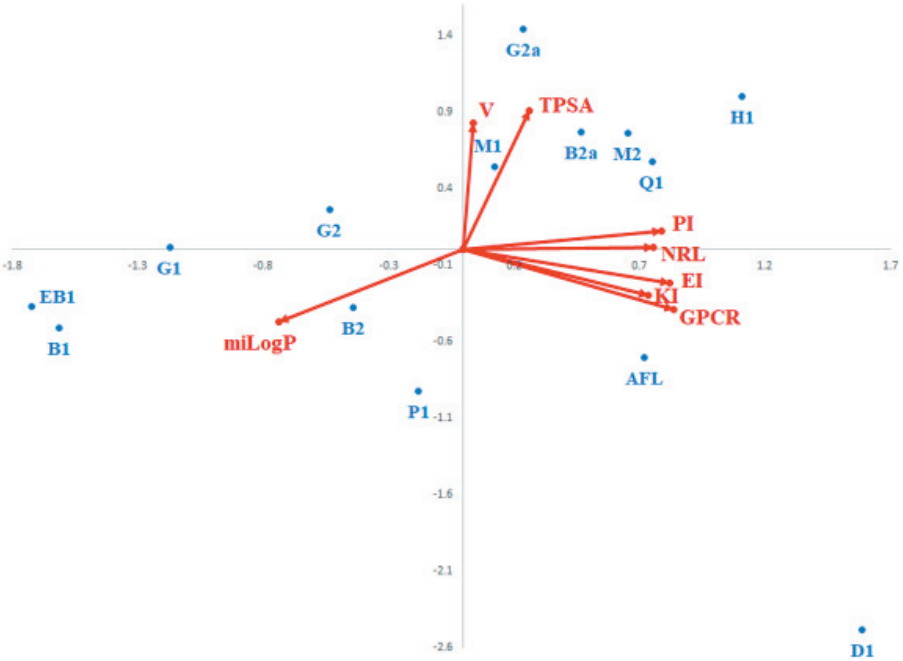
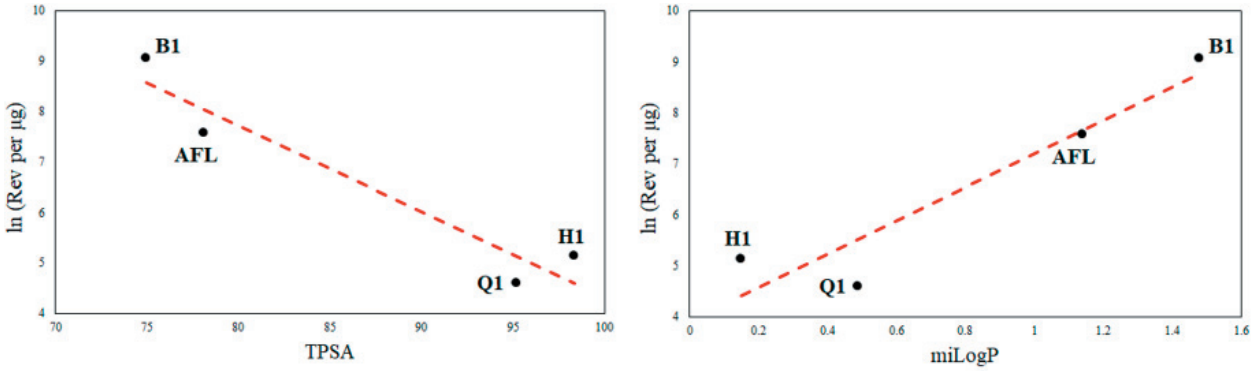


Fig. 2. Correlation between, TPSA, miLogP, GPCR, V, KI, NRL, EI and PI. Red arrows represent correlation vectors.



a) b)

Fig. 3. Dependence of the natural logarithm of the number of revertants on TPSA (a) and miLogP (b).

mental data. There appears to be an interesting correlation with TPSA and miLogP values (curve in Fig. 3), these exponential dependencies are shown in a linearised form for better visualisation. The calculated equations are

$$\ln (\text{Rev}) = -0.1698 \times TPSA + 21.305 \quad (1)$$

$$\ln (\text{Rev}) = 3.2587 \times miLogP + 3.9322 \quad (2)$$

with determination coefficient of 0.9218 for TPSA and 0.8874 for miLogP. Considering linear dependencies for the four parent molecules with experimental data, it is plausible to expect that chemical structures of their mutagenic metabolites are fairly comparable. Unfortunately, the number of points is not sufficient to verify whether this assumption is correct.

Conclusion

To summarise, a systematic QSAR analysis has been carried out during the research. However, to fully confirm the stated results, it is advisable to expand the database of the studied molecules. With more data, the analysis of the quantitative relationship between structure and activity would be more informative. Next, correlations between individual calculated parameters have been determined with the best PCA positive correlation for QSAR inhibition descriptors. On the other hand, miLogP and TPSA parameters correlate negatively. The correlation of experimental data with the TPSA parameter and miLogP are interesting, especially in case of compounds **B1**, **AFL**, **Q1** and **H1**, all of which contain a double bond that may be responsible for the carcinogenic and toxic properties. Future research should concentrate on the characterisation of the chemical structure of aflatoxins and their epoxy metabolites quantum chemically as well as on the reactivity of the mentioned molecules.

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