

VIRTUAL TOXICITY AND DRUG LIKENESS OF NEWLY SYNTHESIZED METHYLXANTHINES WITH N¹ ARYLPIPERAZINE MOIETY

Lily Andonova¹, Maya Georgieva², Alexander Zlatkov³

Abstract: Aiming to obtain preliminary information on the toxicity, stability and pharmacokinetic behavior of a group of 12 methylxanthines, containing an arylpiperazine moiety at N¹, we applied three virtual methods for prediction. The online hazard-screening tool-PBT profiler was used for toxicity evaluation. The pharmacokinetic behavior and drug like properties of the tested compounds were predicted by two online platforms: Molinspiration Cheminformatics and OSIRIS web-based server. The PBT-profiler tool determined, that the investigated compounds are soil persistent, do not bioaccumulate in the food chain and with the exception of the structures containing bulky bi-phenyl substituents all other molecules are of moderate toxicity. All tested compounds meet Lipinski's Rule of Five border conditions and have high values for drug likeness and drug score, which makes them suitable for future optimizations.

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Introduction

Among the purine derivatives, a great number of studies researches are aimed at modifying the structure of methylxanthines because of their diverse biological activity and their well known clinical application as psychostimulants, bronchodilators, diuretics, vasodilators, antiviral and antineoplastic drugs (Boike, 1990; Husain, 1998; Smith, 1995; Zlatkov, 2010). The piperazine nucleus is one of the most important heterocycles exhibiting remarkable pharmacological activities. A slight change in the substitution pattern of the piperazine nucleus causes distinguishable differences in their pharmacological activities such as antipsychotic, anticonvulsant, antiarrhythmic, antimicrobial, antimalarial, citotoxic and antioxidant characteristics (Obniska, 2003; Pietrzycka, 2006). We are interested in the structural combination of these two fragments as a possibility to obtain new derivatives with biological activity.

Of great importance for the use and introduction of newly synthesized molecules as potential drugs is any preliminary information on the toxicity, stability and influence of some structural parameters on the molecule's pharmacokinetic behavior.

Pharmaceuticals are a new type generation of contaminants of emerging concern (CEC) for the environment because they too are frequently overused. Even at very low concentrations certain pharmaceuticals can affect wildlife and ecosystem. Following the necessities and requirements of minimal usage of live animals, any possibility of preliminary identification of toxicity and hazardous properties of new compounds is advisable. The possibility of applying efficient screening systems in order to highlight the compounds predicted as the most hazardous, in particular for the environment is important when using fast and cheap computational tools as good preliminary screening methods to evaluate chemicals for their potential hazards (Sangion, 2016).

Of particular relevance to considering a compound as a potential drug is its beneficial pharmacokinetic behavior in the human organism. The ability to provide the necessary bioavailability, the ability to transport a certain structure to the site of action through different membranes and the optimal metabolism and elimination are key parameters in the development of new potential drugs.

The aim of this paper is the early prediction of pharmacokinetic properties of newly synthesized structures, which would be particularly useful and would save unnecessary test costs for a number of pre-discarded products. In the present work we have applied a virtual evaluation of the toxicity and the two approaches in an attempt to evaluate the drug-like properties: the Lipinski's Rule of Five limitations and the OSIRIS Property Explorer evaluation score.

¹ Medical University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2 Dunav Street, 1000 Sofia, Bulgaria, lilyandonova@gmail.com

² Medical University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2 Dunav Street, 1000 Sofia, Bulgaria, maya.bg77@gmail.com

³ Medical University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2 Dunav Street, 1000 Sofia, Bulgaria, alex.zlatkov@gmail.com

Methods

Calculation of a theoretical PBT-score (Persistence, Bioconcentration potential, and fish chronic Toxicity from chemical structure)

To express the relative amount of concern for Persistence and Bioaccumulation (relative to other substances, or relative to known POPs and/or PBT substances), a quantitative, continuous score is developed, based on properties that can be calculated directly from the structure and are explained in detail elsewhere (Rorije, 2011).

Calculation of Lipinski's Rule of Five parameters

The Lipinski's parameters were calculated with the help of the web based server Molinspiration Cheminformatics (Molinspiration Cheminformatics, 2012, retrieved from www.molinspiration.com).

Calculation of OSIRIS parameters

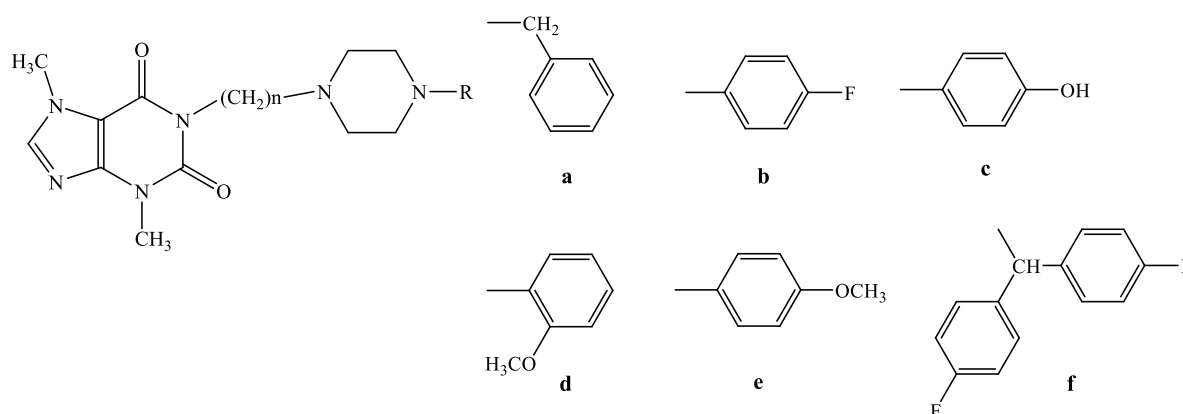
The druglikeness was assessed by calculation of two parameters: Fragment Based Druglikeness and Drug Score included in the OSIRIS property explorer web tool methodology (OSIRIS Property Explorer.; <http://www.organic-chemistry.org/prog/peo/>)

Results and discussion

Virtual evaluation of toxicity.

The evaluation of toxicity is of utmost importance to human health. The prognostic toxicology is an attractive tool for studying the effects on human health as well as assessing environmental risk. The toxic effect of each chemical compound depends on the type, the mode of exposure and the dose. PBT Profiler is an online risk screening tool that predicts the potential of the products to be retained in the environment, to be bioaccumulative in animals and to exhibit toxicity - properties that cause concern for human health and the environment. Moreover, it is important to note that this screening approach can be applied with two different aims: 1) to all existing chemicals, even without experimental data, to rank them and highlight the most potentially hazardous while focusing only on those which would need costly experimental tests, 2) to not yet synthesized molecules, for planning, a theoretical deduction in the chemical design, producing potentially environmentally safer and more sustainable alternatives to those molecules that are identified as potentially hazardous (Cassani, 2015). In the presented research we evaluate the potential risk of a group of 12 methylxanthines, containing an arylpiperazine moiety at N¹ (Scheme 1, series 1, compounds **1a-e** and series 2, compounds **2a-e**) using the online hazard-screening tool-PBT Profiler (<http://www.pbtprofiler.net/>).

Scheme 1: Structures of methylxanthines, containing an arylpiperazine moiety at N¹, obtained by the previously described procedure (Andonova, 2014)



Source: Author

PBT Profiler is a screening server that uses the limits published in the EPA (United States Environmental Protection Agency) to automatically identify compounds that are potentially persistent in the environment and capable of bioaccumulation in the food chain. The results for assessing the stability, bioaccumulation and toxicity of compounds **1a-f** and **2a-f** are presented in Table 1.

The analysis of the data in Table 1 shows that the methylxanthines, containing an arylpiperazine moiety at N¹ are persistent in soil and exhibit chronic fish toxicity (Fish ChV-fish chronic value). PBT Profiler shows that the tested compounds **1a-f** and **2a-f** and the theobromine are not expected to bioaccumulate in the food chain as they do not exceed the BCF (bioconcentration factor) criteria. The majority of the compounds have an average toxicity of 0.1-10 mg/l. The most toxic were compounds **1f** and **2f** for which the fish ChV criteria had values less than 0.1 mg/l.

Table 1: PBT Profiler assessment of the tested compounds.

№	Compound	Stability				Bioaccumulation	Chronic toxicity				
		Half-life (days)		% in any environment		BCF	Fish ChV (mg/l)				
		W	S	Sd	A	W	S	Sd	A		
1	1a	60	120	540	0.071	11%	88%	0%	0%	5.6	0.21
2	1b	180	360	1600	0.083	12%	88%	0%	0%	14	0.11
3	1c	60	120	540	0.067	16%	84%	0%	0%	3	0.33
4	1d	180	360	1600	0.058	20%	80%	0%	0%	12	0.13
5	1e	180	360	1600	0.058	20%	80%	0%	0%	12	0.13
6	1f	180	360	1600	0.067	3%	86%	11%	0%	120	0.033
7	2a	60	120	540	0.071	10%	90%	0%	0%	12	0.13
8	2b	180	360	1600	0.083	9%	91%	0%	0%	30	0.07
9	2c	60	120	540	0.067	13%	87%	0%	0%	6.4	0.2
	2d	180	360	1600	0.058	14%	86%	0%	0%	25	0.081
	2e	180	360	1600	0.058	14%	86%	0%	0%	25	0.081
	2f	180	360	1600	0.067	3%	80%	17%	0%	260	0.02
10	Theobromine	15	30	140	0.83	31%	69%	0%	0%	3.2	0.56

W – water, S-soil, Sd-sediment, A-air

Source: Author

Assessment of drug like properties.

Determination of Lipinski's Rule of Five parameters

Lipinski's rule of five (RO5) is a rule of thumb to evaluate druglikeness or to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans (Ghannay, 2017). The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules (Lipinski, 2001; Lipinski, 2004). The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active. Candidate drugs that confirm to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market (Lipinski, 2004; Leeson, 2007). Lipinski's rule states that, in general, an orally active drug should have no more than one violation of the following criteria: 1) have no more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms); 2) have no more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms); 3) have a molecular mass less than 500 daltons and 4) have an octanol-water partition coefficient logP which is not greater than 5. In the present work, the Lipinski's parameters were calculated with the help of the web based server Molinspiration Cheminformatics (Molinspiration Cheminformatics, 2012, Georgieva, 2014). The obtained results are presented in Table 2. In addition, we determined the extend of absorption, expressed by the percentage of absorption. The absorption percent was calculated using the expression (Zhao, 2002):

$$\%ABS = 109 - 0.345PSA.$$

From the calculated logP values presented in the table, it can be seen that most of the compounds have hydrophobic properties, which would contribute to their good permeability through a cell membrane, respectively, for good absorption. The theoretically determined % ABS values can be considered as confirmation of this conclusion.

From the results we can see that the tested compounds meet Lipinski's border conditions, which makes them suitable for future optimizations. A deviation from the rule is only observed for compounds **1f** and **2f** where the molecular weight is above 500, but this would not have a significant effect on the behavior of these compounds since the parameters do not violate more than one indicator.

Table 2: Parameters of Lipinski's Rule of Five calculated using Molinspiration cheminformatics.

Compound	Mw	LogP	TPSA	nON	nOHNH	n rotb	volume	%ABS
1a	396.50	1.653	68.312	8	0	6	371.62	85.43
1b	400.46	2.116	68.312	8	0	5	359.75	85.43
1c	398.47	1.473	88.54	9	1	5	362.83	78.45
1d	412.94	1.961	77.546	9	0	6	380.36	85.25
1e	412.49	2.009	77.546	9	0	6	380.36	85.25
1f	508.57	3.759	68.312	8	0	7	452.92	85.43
2a	410.52	1.924	68.312	8	0	7	388.42	85.43
2b	414.49	2.386	68.312	8	0	6	376.55	85.43
2c	412.64	1.744	88.54	9	1	6	379.64	78.45
2d	426.52	2.231	77.546	9	0	7	397.16	82.25
2e	426.52	2.279	77.521	9	0	7	397.16	82.26
2f	522.60	4.029	68.312	8	0	8	469.72	85.43
Theobromine	180.8	-0.951	72.693	6	1	0	150.69	83.92

Source: Author

Evaluation of drug-like properties of the tested compounds, using the OSIRIS web-based server.

The corresponding molecule's druglikeness was assessed by two parameters: Fragment Based Druglikeness and Drug Score. (OSIRIS Property Explorer. <http://www.organic-chemistry.org/prog/peo/>, Nadeem, 2016).

Fragment Based Druglikeness.

This parameter is based on a list of about 5300 defined structural fragments with proven drug identity and their grades of drug likeness. In this case, the drug likeness of the molecule being examined is calculated using the equation:

$$d = \frac{\sum v_i}{\sqrt{n}}$$

A positive value states that the analyzed molecule contains predominantly fragments which are frequently present in commercial drugs. However, this does not guarantee that these fragments are well balanced with respect to other important properties, such as the general lipophilicity of the molecule.

Drug Score.

The drug score combines druglikeness, cLogP, logS, molecular weight and toxicity risks in one useful value that may be used to judge the compound's overall potential to qualify for a drug. This value is calculated by multiplying contributions of the individual properties with the first equation:

$$ds = \Pi \left(\frac{1}{2} + \frac{1}{2} S_i \right) \cdot \Pi t_i$$

where: ds is the drug score. s_i are the contributions calculated directly from cLogP, logS, molweight and druglikeness (p_i) via the second equation which describes a spline curve.

$$s = \frac{1}{1 + e^{ap+b}}$$

Parameters a and b are (1, -5), (1, 5), (0.012, -6) and (1, 0) for cLogP, logS, molweight and druglikeness, respectively. t_i are the contributions taken from the 4 toxicity risk types. The t_i values are 1.0, 0.8 and 0.6 for *no risk*, *medium risk* and *high risk*, respectively (<http://www.organic-chemistry.org/prog/peo/>).

chemistry.org/prog/peo/drugScore.html). The corresponding values of the computational analysis are presented in Table 3.

Table 3: Parameters Druglikeness and Drug-score for compounds 1a-f и 2a-f , calculated using OSIRIS Property Explorer.									
№	Toxicity risk				cLogP	Solu- bility	Mw	Drug- likeness	Drug- score
	Muta- genicity	Oncogenicity	Irritant	Repro- ductive effect					
1a	1	1	1	1	1.47	-1.73	396	9.48	0.86
1b	1	1	1	1	1.65	-2.57	400	7.65	0.83
1c	1	1	1	1	1.20	-1.96	398	8.9	0.90
1d	1	1	1	1	1.48	-2.27	412	10.29	0.83
1e	1	1	1	1	1.48	-2.27	412	7.47	0.83
1f	1	1	1	1	3.39	-3.62	508	9.3	0.61
2a	1	1	1	1	1.93	-2.0	410	8.11	0.83
2b	1	1	1	1	2.1	-2.84	414	6.27	0.80
2c	1	1	1	1	1.56	-2.23	412	7.52	0.83
2d	1	1	1	1	1.93	-2.54	426	8.94	0.8
2e	1	1	1	1	1.93	-2.54	426	6.1	0.8
2f	1	1	1	1	3.84	-3.89	522	7.95	0.55
Theo- bromine	0.6	0.6	1	0.6	-0.43	-1.51	180	3.42	0.21

Source: Author

From the obtained results, it can be seen that the studied derivatives all have high positive values for the **Druglikeness** parameter and close to 1.0 values for the **Drug Score** parameter. The highest value for **Drug Score** holds compound **1c**. For all of the tested compounds, there was a risk assessment of the four types of toxicity observed ($t_i = 1.0$ for compounds **1a-f** and **2a-f**).

Conclusion

As a summary of the obtained results, we can state that using the PBT profiler server for the studied N^1 arylpiperazine containing methylxanthines, was found to be very persistent in the environment with the most pronounced soil resistance. However, they do not bioaccumulate in the food chain and with the exception of the compounds with bulk biphenyl substituents in their structure (**1f** and **2f**) which exhibited high toxicity in the food chain; all the others had only moderate toxicity.

From the analysis of the obtained results, it can be concluded that the introduction of the bulky bifluorophenyl methyl substituent in the structure of the newly synthesized compounds (**1f** and **2f**) leads to a decrease in the **Drug score** parameter. These compounds also have the largest molecular volume and molecular mass above 500 (deviation from Lipinski's Rule 5), which is probably the main reason for a decrease in the values for the **Drug Score** parameter. As shown, the determined molecular properties of these compounds justify their usage as potential leading molecules for drug design.

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