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Molecular modification proposal: analysis of physicochemical, pharmacokinetic and toxicological parameters "*in silico*" of barbituric drugs

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ABSTRACT
This research aimed to propose molecular modifications and compare the characteristics of three barbiturate drugs and their analogues developed in silico through procedures and computational techniques using free programs. Barbiturates were the class chosen for this study, the selected drugs were: Phenobarbital, Pentobarbital and Amobarbital, being modified through simulations in silico. Three analogues were proposed, being analyzed and compared with the prototypes, using the free programs, Marvinsketch, where it was possible to evaluate parameters such as: Log P, hydrogen donors/acceptors and Van Der Walls Surface. In PreADMET, parameters such as BHE, HIA, Caco-2, MDCK, CYP-3A4, Ames Test, Carcinogenicity (Rats) and hERG Risk were obtained. Through the Osiris Property Explorer, the following parameters were evaluated: Mutagenicity, tumorigenicity, irritability, reproductive effect, solubility, molecular weight, polarity, druglikeness and Drug-Score. With the results obtained in PreADMET referring to BHE, the analogues BBT-F and BBT-A obtained better results, while the BBT-P had a worsening in the result. In relation to HIA, the values proved to be satisfactory, exhibiting good intestinal absorption. As for toxicity, the Ames test was verified, where all molecules were mutagenic. With the results obtained in Osiris, all the prototype drugs proved to be mutagenic, while in the analogues, only BBT-P showed mutagenicity. As for tumorigenicity, the prototype drugs remained at medium to high risk, while analogue drugs all showed non-tumorigenic results. Therefore, it is concluded that it was possible to make a preliminary, fast and low-cost analysis, helping in the research of new drugs through computational tools.

Keywords: Computational techniques, analogues, molecule, toxicity.

1 INTRODUCTION

In 1864, the combination made by Adolf von Baeyer, of urea and malonic acid found in apples using condensation, would discover barbituric acid, which in the future substances derived from this acid would be used as medicines, being depressants of the central nervous system (CNS) capable ofproducing sedative and anxiolytic effects, however, in slightly higher doses are able to cause death by respiratory depression and cardiovascular insufficiency (Morales, 2019).

Molecular modification aims to improve the usefulness of the drug by altering its previously known chemical structure (Dos Santos, et al., 2018). This technique, in turn, is widely used for the development of analogous structures, seeking better pharmacokinetics and/or pharmacodynamics and

similar interactions with receptors, and may even have unexpected reactions in other receptors (Pereira, 2022).

The pharmaceutical industry is always looking for innovations, being dynamic and always very active in research and development (Aguiar, et al., 2020). With the constant need for innovations, the pharmaceutical industry constantly seeks to introduce new products, services and processes or in the improvement of existing ones, with their functionalities or characteristics (Melo, 2019).

In *silicon* experiments are computational tests that use software to collect and analyze physical-chemical, biological and medical data. Through *in silic* pharmacology it is possible to integrate these computational techniques, and it is possible to create models and simulations capable of providing relevant predictions and even therapeutic medical hypotheses (Dos Santos, et al., 2018). In addition to *in vivo* and in *vitro* studies, in *silic* experiments have been increasingly requested due to their low cost and simplification in drug development. In *silic* study has been widely employed to discover new substances with therapeutic potential, as well as in the elimination of candidates without clinical efficacy, high toxicity, many adverse reactions and inadequate pharmacokinetic properties (Bernardo, 2022).

In view of the above, this study aimed to propose molecular modifications and compare the characteristics of three barbiturate drugs and their analogues developed in *silic* through procedures and computational techniques used in the development of drugs using free programs.

2 METHODOLOGIES

According to Pereira et al. (2018), This is an experimental, qualitative, quantitative and explanatory research, based on the study of the pharmacokinetic, toxicological and physicochemical characteristics of prototypes of barbiturate anxiolytic drugs that are Phenobarbital, Pentobarbital and Amobarbital, a molecular modification was proposedfor each molecule, the six molecules were evaluated and compared *in silic* through the use of free software of Medicinal Pharmaceutical Chemistry that were used for data collection and analysis.

Barbituratewere the class chosen for this study because there is a large margin of adverse effects and their need for control of use, because it has a high level of toxicity, in view of this were modified through in *silic* simulations that is directlyrelated to the biological terms better known as in *vivo* and *in vitro,* where computer programs are used that can be used to make predictions about the outcome of the changes made. All survey data was collected during the year2020.

2.1 MARVIN SKETCH 6.2.2

The tools of Marvin Sketch were used to generate the chemical structures of the molecules, the

number of possible stereoisomers was calculated and the isomery of the molecule was observed, in addition to generating the IUPAC name of the compounds, the images of the molecules optimized in 2D were generated, after that the hydrogens were added and all the molecules in 3D were optimized and calculations were performed for the physicochemical analyses, log P, and donors and acceptors of Hydrogen of all six molecules in the study (prototypes and analogues).

2.2 PreADMET 2.1

The online software PreADMET was used to obtain data related to toxicity, pharmacokinetics (absorption, distribution, metabolism and excretion) of the prototype drugs and their analogues, through quantitative analysis. The following parameters were analyzed, blood-brain barrier absorption capacity (BBB), intestinal absorption of drugs in humans (HIA), Caco-2 which is very valuable to estimate the absorption index of compounds by oral administration and MDCK (Madin-Darby canine kid ney) which refers to the excretion of drugs. Regarding the metabolization of drugs, the non-inhibition capacity of prototypes and analogues was also measured through the behavior of CYP-3A4, which is a subfamily of CYP-450.

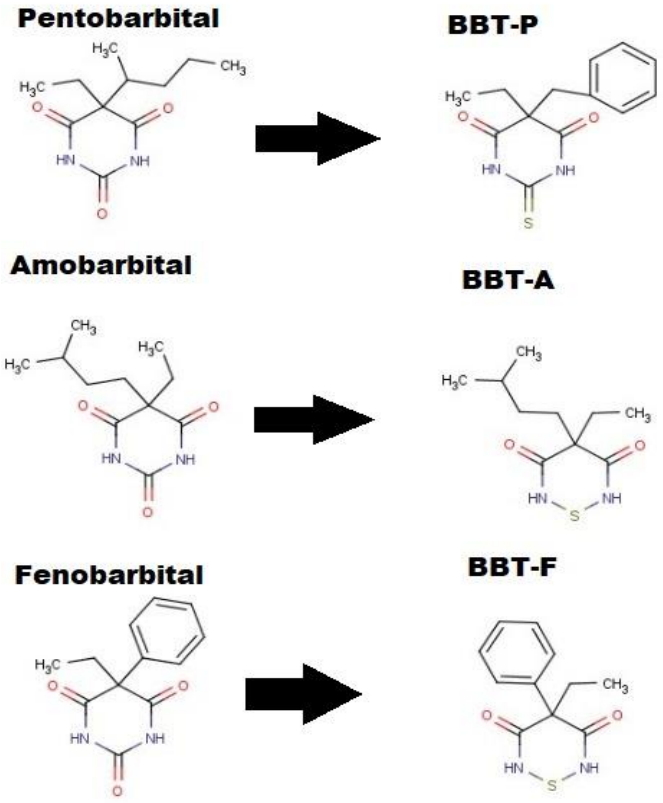
2.3 OSIRIS PROPERTY EXPLORER

TheOSIRIS Property Explorer programme was the tool used to promote molecular modifications. Through it it was possible to observe some physico-chemical parameters and also about undesirable effects, important for the improvement of prototype fabrics. Another parameter evaluated was the automatic prediction of mutagenicity, toxicity, irritability, reproductive effect, Log P, Solubility, molecular weight, polarity, druglikeness and Drug-Score. The results achieved by their predictions were evaluated and coded through the colors red, yellow and green that refer to the risks of the drugs, thus considering the color green indicates performance compatible with drugs that have potential and safetyfor therapeutic use.

3 RESULTS AND DISCUSSION

It was carried out in the initial stage the assembly of images of the 2D structures, where it is possible to verify the molecular changes that were made from the prototypes, and the analogues were all named. The first modified drug received the acronym BBT-A (obtained from the Amobarbital prototype), BBT-P (obtained from Pentobarbital) and BBT-F (obtained from Phenobarbital). All of these can be seen in the following image (figure 1).

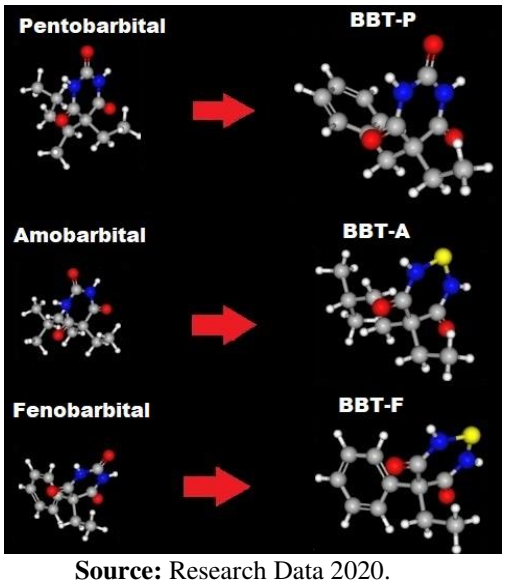
Figure 1 – 2D schematic of the molecules of the prototype drugs and analogues developed through Marvin Sketch 6.2.2



Source: Survey Data 2020.

In Figure 2 is shown the arrangement of the molecules as well as in Figure 1, but being represented in 3D where it is also possible to observe the prototypes on the left side and the analogues on the right side.

Figure 2 – 3D schematic of the molecules of the prototype drugs and analogues developed through Marvin Sketch 6. 2.2.



Source: Research Data 2020.

A study conducted in 1997, by Christopher A. Lipinski and collaborators of the pharmaceutical industry Pfizer, using more than 2000 drugs where they evaluated the main physicochemical properties that the drugs needed to have good intestinal permeability and solubility in water, which are indispensable for oral use. And going into the study of Lipinski's rule of 5, 5 essential properties for molecules such as log P, molecular weight, acetors and hydrogen donors (H), and the interaction sites for the main drug targets are highlighted (Santos, Gonsalves & Araujo, 2018).

Using the Marvin Sketch software, it was possible to obtain the values of table 1, all the mass values obtained were similar, on average of 235.58 Daltons for both prototypes and analogues. According to Lipinski's rule (rule of 5), the ideal mass number for drugs has to be up to a maximum of 500 Daltons in order for them to be able to cross the cellular branes of the organism. (Lipinski, et al., 1997) Based on this rule, all molecules presented in table 1 are within the requirements established by the rule.

Another physicochemical parameter evaluated was the Log P, which represents the partition coefficient, in order to quantify the lipophilicity of bioactive compounds. According to the rule of 5, the ideal values for Log P are between 1 and 5, where values below 1 represent low ability to cross cell membranes. When they are above 5, they represent a high capacity of the molecule to present toxicity, because it will cross the cell membranes in an exaggerated way and can cause several problems for individuals. According to the results obtained (table 1) the values are within the established parameters.

The rule of 5 dictates that the ideal number for (H) donors that a molecule must contain is at most 5, while the acceptors of (H) that can contain in the molecule is at most 10. The greater or lesser the number of donors or acceptors, the molecule may have greater or lesser interaction with molecules of H_2What are in the body, because if the molecule has many interactions it may end up affecting the absorption process. The results obtained (table 1) are within what was proposed by the rule.

Table 1 – Research of physicochemical parameters based on Lipinski's rule obtained in Marvin Sketch 6.2.2

Drug	Dough	Log P	Donors of H	H acceptors	Van der Waals surface
Amobarbital	226,2	1.89	2	3	369,2
BBT-A	230,3	2,37	2	2	362,0
Pentobarbital	226,2	1,86	2	3	367,9
BBT-P	262,3	2,69	2	2	357,3
Phenobarbital	232,2	1,41	2	3	322,3
BBT-F	236,3	2,07	2	2	315,1

Source: Research data 2020.

The results obtained based on PreADMET were satisfactory, these can be seen in the following table (Table 2).

Table 2 – List of pharmacokinetic parameters found through PreADMET 2.1 data.

Drug	BHE	HIA	Kermit-2	CYP3A4	MDCK	LPP
Amobarbital	1.01	82.1	1.29	No	75.1	81.0
BBT-A	1.39	84.4	19.5	No	68.6	83.7
Pentobarbital	1.01	82.1	1.29	No	61.3	80.9
BBT-P	0.93	93.9	3.08	No	33.9	83.6
Phenobarbital	0.49	89.2	2.0	No	36.4	85.2
BBT-F	0.64	88.2	20.1	No	31.4	56.2

Source: Research Data 2020.

The results obtained refer to the BHE that is a barrier of the central nervous system that acts making it difficult for substances from the blood to access the central nervous system (Vieira & Sousa, 2013). The drugs BBT-F and BBT-A showed the best results in the blood-brain barrier in relation to their prototypes, while the analogue BBT-P showed a decrease.

The values used to define human intestinal absorption are dictated by the HIA parameter which are: high absorption of 70% to 100%, regular absorption of 20% to 70% and low absorption of 0% to 20%. (Da Silva Miranda, Salazar & de Brito, 2021). The values related to HIA were satisfactory in all drugs, that is, they presented a good intestinal absorption.

Caco-2 are a cell line of the intestine, and it is an important parameter referring to the intestinal absorption of drugs, if the permeability of the molecules is below the expected values, they will hardly have a satisfactory absorption (Gabriel, 2021). The best results were observed in the analogues BBT-F and BBT-A, presenting a greater capacity of absorption by a specific cell line of the human colon.

CYP3A4 belongs to the CYP450 subfamily and is important for hepatic drug metabolism in adult humans and some drugs may inhibit this enzyme (Habenschus, 2021). The results obtained in PreADMET 2.1. in Table 2, we can observe that none of the drugs had an action on this enzyme.

MDCK are cells ofcanine r im origin, and have been used to predict the absorption of compounds since 1989, however, through this parameter it is possible to measure the renal excretion of substances (Dolabela, et al., 2018). According to table 2, it can be observed that there was a decrease in allanalogues in relation to prototypes, which results in a lower excretion of drugs.

LPP refers to the binding of drugs to plasma proteins, this parameter disposes of the ability of drugs to remain bound to plasma proteins and increasetheir availability in the body (Da Silva Miranda, Salazar & de Brito, 2021). In the results obtained in table 2, the analogues BBT-A and BBT-P had a small increase in LPP in relation to the Amobarbital and Pentobarbital prototypes, demonstrating a greater ability of the drug to remain bound in the reserve form and increase its availability in the body, while BBT-F had a large decrease in this parameter in relation to its Phenobarbital prototype, meaning that it has a larger free fraction relative to its prototype.

Table 3 – List of toxicological parameters found through preadmet 2.1 data.

Drug	Ames Test	Carcinogenicity (Rats)	hERG Risk
Amobarbital	Mutagenic	Positive	Low
BBT-A	Mutagenic	Negative	Low
Pentobarbital	Mutagenic	Positive	Low
BBT-P	Mutagenic	Negative	Medium
Phenobarbital	Mutagenic	Positive	Medium
BBT-F	Mutagenic	Negative	Medium

Source: Research data 2020.

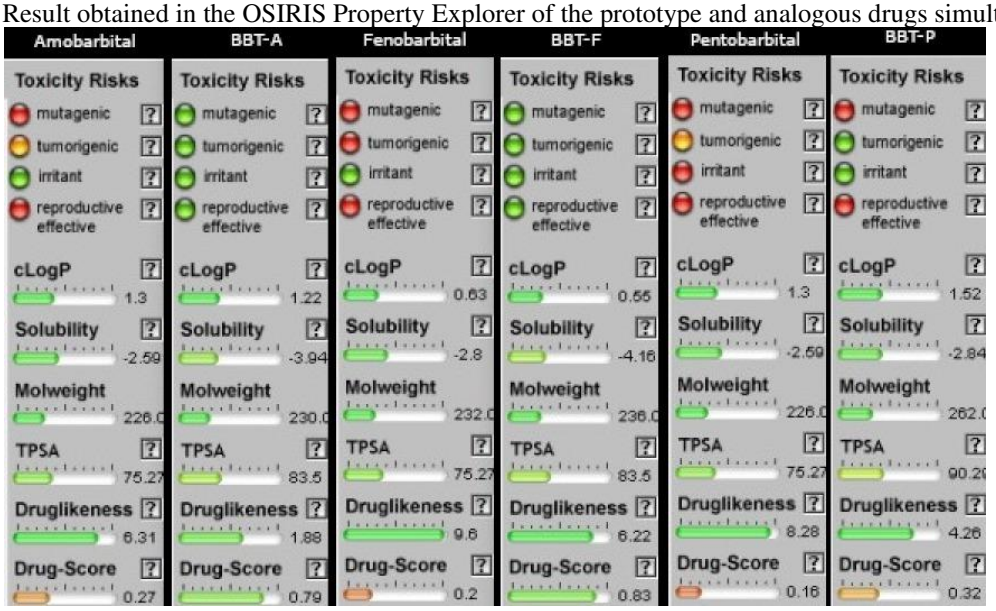
As for the parameters related to toxicity, the Ames test was verified, it is done through the use of bacterial strains, measuring the mutagenicity of the molecules (Mochiutti, et al., 2019). Based on the PreAdmet results evidenced by table 3, all molecules were shown to be mutagenicin relation to the Ames test.

With respect to carcinogenicity, it is the ability of substances to promote cancer in rats or mice (Pimentel, et al., 2017). The carcinogenicity result of PreADMET applies an algorithm derived from data from the NTP (National Toxicology Program) and the Food and Drug Administration - USA (Vieira et al., 2014). All results were satisfactory in relation to analogues, as they presented negative results in carcinogenicity for rats, while the prototypes all had positive results in carcinogenicity in rats.

The protein encoded by the acronym hERG corresponds to a channel of selective cardiac potassium ions (Santana, Miranda & Sousa, 2020). Table 3 shows the risk thateach molecule can cause in the inhibition of this protein, thus generating cardiovascular problems. The prototypes Amobarbital and Pentobarbital presented low risk for inhibition of this protein, the others presented medium risk. On the other hand, the analogues with the exceptionof BBT-A presented low risk, the others presented a medium risk for the inhibition capacity of hERG.

Figure 3 denotes important results referring to different degrees of toxic risks in all molecules found in the Osiris, followedby the color system, red, yellow and green, further down, you can observe the physicochemical parameters, where all parameters will be evaluated and compared next.

Figure 3 – Result obtained in the OSIRIS Property Explorer of the prototype and analogous drugs simultaneously.



Source: Research Data. 2020

In Figure 2 the results obtained in Osiris, first show the risks related to mutagenicity, where in all prototype drugs are in red, thus denoting high mutagenic risk, in relation to analogous drugs we have the results in green for BBT-A and BBT-F, which means to be within the non-mutagenic parameters, the BBT-F remained in red. Regarding tumorigenicity, the protódrugs remained between yellow, which represents medium risk, and red. While the analogous drugs, all showed results in green, which means that in these molecules there is no tumorigenic component. As for the irritating effect, Pentobarbital was presented in red, while all other prototypes and analogues were in green. Regarding the reproductive effect, which is the effect on the capacity of human reproduction, all prototypes presented results and m red, while the analogues BBT-A and BBT-F, obtained results in green and BBT-P in red.

Regarding Log P, which refers to lipophilicity, all analogues obtained an increase with the exception of BBT-A. As for solubility, the analogues were somewhat unfavorable in relation to the prototypes. Regarding molecular weight and polarity (TPSA), all analogues obtained an improvement in the result compared to the prototypes. Regarding druglikeness, which measures the degree of similarity with other drugs, all of them were similar to other drugs used in the therapy. The parameter that measures the drug-score, the analogues showed a great improvement with the exception of BBT-P, which had only a small improvement compared to its prototype.

4 CONCLUSIONS

Therefore, it is concluded that it was possible to make a preliminary, fast and low-cost analysis, assisting in the research of new drugs through computational tools. Successfully evaluating the modifications and prototype drugs, among them thephysico-chemical, pharmacokinetic and

toxicological in *silic* properties, being possible to elucidate beneficial and similar results in such molecules, however it is necessary studies such as molecular docking and in *vitro* and *in vivo* assays, to evaluate possibleadverse effects, mechanisms of action and complementary characteristics.

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