Synthesis, antineoplastic activity and *in silico* studies of (*S*)-*N*-(1-hydroxy-3-methylbutan-2-yl)-3-(*p*-tolyl)-1,2,4-oxadiazole-5-carboxamide

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Abstract

The development of chemotherapy agents without side effects is a major challenge, since traditional medicines usually have undesirable properties such as high toxicity, resistance and low bioavailability. In this sense, computational methods play a crucial role in the discovery and optimization of new drugs, as they combine speed and efficiency with low cost. The 1,2,4-oxadiazoles are one of the main classes of heterocyclics due to their numerous biological applications.

In this work, we report the synthesis, antineoplastic evaluation and in silico study of a new 1,2,4-oxadiazole. The (S)-N-(1-hydroxy-3-methylbutan-2-yl)-3-(p-toluyl)-1,2,4-oxadiazole-5-carboxamide was obtained after two reaction steps in excellent yield. Although it has shown low activity in relation to the MCF-7, HCT116 and HL60 tumor cell lines, the molecular docking study indicates that this compound acts in the colchicine site and can inhibit tubulin polymerization. From the calculation of pharmacokinetic properties by the SwissADME and Osiris Property Explorer programs, it is possible to infer that the compound meets the Lipinski rules presenting good oral bioavailability and low toxicity.

Keywords: 1,2,4-oxadiazole, Anticancer, Molecular Docking.

Introduction

The cancer or malignant neoplasm is the abnormal and uncontrollable growth of malignant cells thet invade tissues and organs and can spread by the process of metastasis to other body regions[7]. The World Health Organization[49] estimates that one in five men and one in six women develops cancer in their lifetime.

More than 18.1 million new cancer cases will be diagnosed in 2018 leading to 9.6 million deaths worldwide as per reports[14,49,52]. Despite all the scientific and technological advances in medicine, cancer is still a disease that causes more fear in society, it has become stigma of death and pain[2]. There are many types of cancer treatments that depend on the type of cancer and the severity of the disease. The most common cancer treatments are surgery, radiationtherapy, chemotherapy or a combination of these techniques[32,40].

Chemotherapy is the method (usually oral or intravenous) that uses chemical compounds (chemotherapeutic). The primary goal of chemotherapy is to destroy neoplastic cells, preserving normal ones, but most chemotherapeutic agents act in a non-specific way, damaging both malignant and normal cells, especially the rapid growth of cells in gastrointestinal, capillary and immune system. This explains most of the side effects of chemotherapy: nausea, hair loss and increased susceptibility to infections. Thus, clinical use of these drugs requires that the benefits be confronted with their toxicity in search of a favorable therapeutic result[40].

Cell cycle inhibitors or modulators that interrupt uncontrollable tumor growth are considered highly promising for antineoplastics activities[28,45]. In this respect, tubulin, a protein present in all eukaryotic cells, has a fundamental role. Tubulin was initially identified as the target of colchicine, an alkaloid capable of inhibiting mitosis in mammals[11]. Tubulin consists of two subunits: α-tubulin and β-tubulin which are composed of 450 and 455 amino acids respectively[39]. The interaction of these proteins generates polar cylinders called microtubules which regulate cell division, cell migration and intracellular transport[48]. Thus, drugs that prevent microtubules from performing their primary functions end up leading to mitotic arrest and death of tumor cells by apoptosis[38].

In the last decades, numerous organic molecules have been identified as antimitotic agents that bind to the colchicine site inhibiting tubulin polymerization as is the case of paclitaxel, docetaxel, vinblastine, vincristine, eribulin mesylate and ixabepilone[18]. Although these chemotherapeutic agents have been widely used in the treatment of several types of cancer, they have some disadvantages, namely, high toxicity, development of drug resistance, side effects, low solubility, low oral bioavailability and complex synthesis. Thus, the discovery and development of new antitubulin agents are urgent[28,48].

Recently, some 1,2,4-oxadiazol derivatives were identified as tubulin inhibitors[30,36,51]. The 1,2,4-oxadiazole ring is a heterocyclic structure present in several drugs, to mention, Azilsartan (treatment of hypertension), Prenoxdiazine (cough suppressant) and e Atalurene (Duchenne muscular dystrophy treatment) (Figure 1). The 1,2,4-oxadiazoles are stable in an aqueous medium and have the ability to form various non-covalent interactions such as hydrogen bonds, van der Waals forces and dipole-dipole bonds with different biological targets[35,50]. In addition, oxadiazoles rings are commonly used as bio-isomeric substitutes for esters, amides, carbamates and hydroxamics esters[35,43]. These features have allowed a wide applicability of these compounds in medicinal chemistry, as antitumor[30,36], anti-inflammatory[15], neuroprotectors[21], antihypertensives[53] and antivirals[5] among others[35].

Based on these considerations, the objective of this work was to carry out the synthesis and cytotoxic evaluation of (*S*)-*N*-(1-hydroxy-3-methylbutan-2-yl)-3-(*p*-toluyl)-1,2,4-oxadiazole-5-carboxamide against cancer cells HL60 (Promyelocytic Leukemia), HCT 119 (colon carcinoma - human) and MCF-7 (Breast carcinoma - human). To assess tubulin inhibition, molecular docking was performed at the conchichina binding site. Pharmacokinetic and toxicological properties of this compound have been predicted using an *in silico* approach.

Material and Methods

Reagents and equipment: The reagents and solvents used were obtained in the commercial form of Merck and Sigma-Aldrich, the hexane and ethyl acetate were purified by distillation in Vigreaux column. The reactions were monitored by thin-layer chromatography (LCD) using silica gel plates containing fluorescent indicator F_{254}. The TLC plates were visualized using an ultraviolet (UV) lamp with wavelengths between 254 and 365 nm.

The purification step of the synthesized 1,2,4-oxadiazoles was carried out using liquid chromatography on a glass column using silica gel 60 Merck (70-230 mesh) as the stationary phase and as the mobile phase the solvents hexane and ethyl acetate in different proportions of systems.

The reaction mixtures were evaporated on a Büchi Rotavapor rotary evaporator model R-114 connected to a vacuum pump model KNF Neuberger. The infrared (IR) was performed on an infrared spectrophotometer with Fourier Spectrum 400 FT-IR/FT-NIR Spectrometer model Perkin Elmer. The transmission spectrum IV was obtained with 4 cm^{-1} resolution, 4000 to 400 cm^{-1} spectral region and 40 scans. The sample was subjected to IR analysis prepared as KBr tablets. The sample was prepared at a concentration of 1 g/mL (in AcOEt) in 1 mL cuvette. The heated stir plate and the heating mantle were Fisaton model 754A and 102E respectively.

The characterization of the structures of the compounds was performed using the nuclear magnetic resonance technique of hydrogen (^{1}H NMR) and carbon (^{13}C NMR) in a Varian Unity Plus spectrometer, whose frequency for the hydrogen core was 400 or 300 MHz and for the carbon core it was 100 or 75 MHz. The analyzes were performed using $CDCl_3$ as solvent. The chemical shifts were expressed in δ (ppm) and the coupling constants (*J*) in Hertz (Hz) in relation to the central peak of $CDCl_3$ (7.27) for the ^{1}H NMR spectrum and for the ^{13}C NMR spectrum; the displacements were obtained in relation to the central peaks of $CDCl_3$ (77.0). The device was calibrated using $Si(CH_3)_4$ (0.0 ppm) as an external reference in the case of ^{1}H and ^{13}C NMR.

Experimental Procedure for Synthesis of Compounds: The ethyl 3-(*p*-toluyl)-1,2,4-oxadiazole-5-carboxylate (3) was obtained according to the protocol of Bretanha et al.[6] Thus, to a solution of (*Z*)-*N*-hydroxy-4-methylbenzimidamide (1) (150.08 mg; 1.0 mmol) in ethyl acetate (2.0 ml) was added ethyl chloroxoacetate (2) (150.15 mg; 1.1 mmol).

The reaction mixture was irradiated with ultrasound and maintained at a temperature of 55±5 °C for 3.0 h (the completion of the reaction was confirmed by TLC). After that time, the product was extracted with ethyl acetate and then the solvent was removed under reduced pressure. The 3-(*p*-toluyl)-1,2,4-oxadiazole-5-carboxylate was purified on a chromatographic column using a hexane/ethyl acetate elution system (95:5; v:v).

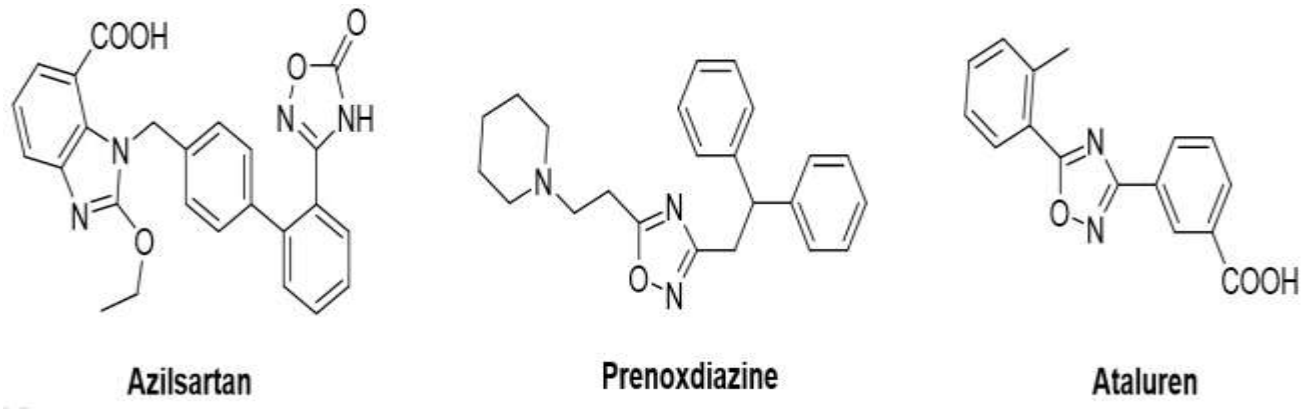


Figure 1: Estructure of Azilsartan, Prenoxdiazine and e Ataluren

The (*S*)-*N*'-(1-hydroxy-3-methylbutan-2-yl)-3-(*p*-toluyl)-1,2,4-oxadiazole-5-carboxamide (5) was obtained from the protocol of Cosimelli et al[8] with some modifications. Thus, 3-(*p*-toluyl) -1,2,4-oxadiazole-5-carboxylate (3) (92.83 mg; 0.4 mmol) was added to a round-bottom flask, the (*S*)-(+)-2-amino-3-methyl-1-butanol (4) (61.86 mg; 0.6 mmol) and chloroform (10 mL). Then, the reaction mixture was left under stirring and heating (60 °C) for 1.0 h (the completion of the reaction was confirmed by TLC). After that time, the product was extracted with ethyl acetate and then the solvent was removed under reduced pressure. The (*S*)-*N*-(1-hydroxy-3-methylbutan-2-yl)-3-(*p*-toluyl)-1,2,4-oxadiazole -5-carboxamide (5) was purified on a chromatographic column using a hexane/ethyl acetate elution system (70:30; v:v).

Evaluation of Cytotoxic Activity: The cytotoxic potential of 1,2,4-oxadiazoles derivatives was realized by the MTT method {3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium} using three cell lines, HL60 (Promyelocytic Leukemia), HCT 119 (colon carcinoma - human) and MCF-7 (Breast carcinoma - human), obtained from the Rio de Janeiro Cell Bank. The test consists of a colorimetric analysis based on the conversion of the yellow salt of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to formazan blue described by Mosmann[31] by the activity of the enzyme succinyl dehydrogenase present in the mitochondria of the viable cell, thus allowing to quantify the percentage of living cells.

The strains were plated at a concentration of 1 x 10^5 cells/mL. The substances previously dissolved in DMSO were serially diluted in the RPMI medium to obtain the final concentrations and added to a 96-well plate. The plates were incubated for 72 hours in an oven at 5% CO_2 at 37ºC. Then, 25 µL of the MTT solution (5 mg/mL) was added and the plates were incubated for 3 hours. The absorbance was read after dissolving the precipitate with pure DMSO in a 595 nm plate spectrophotometer.

Computational studies

Molecular docking: The molecular docking studies were performed using the AutoDock 4.2.6 program. The three-dimensional structures of the compounds were built using Avogadro 1.2.0 software[19] and fully optimized by the semi-empirical method PM6[44] implemented in MOPAC2016 (http://openmopac.net). The ".pdb" binders were prepared for docking by merging the non-polar hydrogens with the corresponding carbon, calculating the partial charge of the atoms using the Gasteiger procedure and defining the rotatable binder connections from the package AutoDockTools-1.5.6.

The tubulin was chosen as a pharmacological target for molecular docking studies due to its relevance as an anti-tumor target for a wide range of drugs used clinically. Its crystallized structure was recovered in the RCSB Protein Data Bank with ID: 4O2B. The colchicine was chosen as the reference drug for molecular docking studies. The Lamarckian genetic algorithm was used in AutoDock 4.2.6 to determine the best conformations and orientations of the ligands.

Through the grid box, the anchorage location in the protein structure of tubulin (4O2B) was defined for molecular docking calculations, using the enzyme's co-crystallized ligand in the RCSB Protein Data Bank, the colchicine, as the center of the box large enough to reach the amino acids belonging to the active site[37]. The resulting coupling poses as well as images showing enzyme-ligand interactions were generated and analyzed using AutoDockTools, BIOVIA Discovery Studio Visualizer and Visual Molecular Dynamics[20].

Pharmacokinetic, toxicological and chemical parameters *in silico*: The values of consensual Log $P_{o/w}$ (cLogP), molecular mass (MM), N° of hydrogen bond donors (nHBD), N° of hydrogen bond acceptors (nHBA), N° of Lipinski rule violations, gastrointestinal absorption and Leadlikeness were obtained through the SwissADME digital bioinformatics platform[10].

Through the Osiris Property Explorer program (https://www.organic-chemistry.org/prog/peo/), information was obtained regarding the chronic toxicity (CT) of compound 5 with the result being classified in the interface of the program through colors where the red color indicates high risk, the yellow color moderate risk and the green color low risk. The calculated values of Druglikeness and Drug score were also obtained from the same program.

Results

The general strategy for the synthesis of the new (*S*)-*N*-(1-hydroxy-3-methylbutan-2-yl)-3-(*p*-toluyl)-1,2,4-oxadiazole -5-carboxamide (5) is described in figure 2. Thus, (*Z*)-*N*-hydroxy-4-methylbenzimidamide (1) was subjected to successive reactions as the reactions of *O*-acylation followed by cyclodehydration and aminolysis.

The ethyl 3-(*p*-toluyl)-1,2,4-oxadiazole-5-carboxylate (3) was obtained as a white amorphous solid with 72% yield after 3.0 hours of reaction. The formation of compound occurred initially by attacking the hydroxyl (stronger nucleophile) of (*Z*)-*N*-hydroxy-4-methylbenzimidamide (1) to acyl chloride (region of the molecule most deficient in electrons) of ethyl chloroxoacetate (2) called *O*-acylation followed by thermal intramolecular cyclization with the elimination of water from the formed intermediate.

It is worth mentioning that the formation of the 3,5-diaryl-1,2,4-oxadiazole dimer was not observed as reported by some authors[13,33] due to the thermal homo-coupling of two molecules of (*Z*)-*N*'-hydroxy-4-methylbenzimidamide (1).

The compound 5 was obtained in the short reaction time (1.0 h) in 92% yield as an amorphous white solid with m.p.

125.5-126.0 °C. Its structure was confirmed by different techniques of structural analysis of organic compounds. For example, the polarimetric analysis, a sensitive and non-destructive technique provided a rotation angle of polarized $[\alpha]_D^{25}$ -19.4 (c 1.0 mg/mL, EtOAc) indicating the presence of a chiral center in compound 5. The spectroscopic data from the absorption benches in the infrared region and chemical displacement of the hydrogen and carbon nuclei of compound 5 are in accordance with the proposed structure.

For a better understanding of the biological characteristics of compounds 3 and 5, these oxadiazoles were subjected to the evaluation of their antineoplastic properties by the MTT method[31] to obtain their respective cell growth inhibition percentages against the initial concentration of 25 µg/mL. For this, tumor cell lines MCF-7, HCT 116 and HL 60 were selected due to the significant epidemiological importance of these cells in cancer cases[42]. The results are shown in table 1. For all tumor cell lines analyzed, compound 5 was more active than its synthetic precursor compound 3 showing inhibition values that reached 31.16% for human breast cancer cells (MCF-7). These results indicate that the structural modification in compound 3 improved the antineoplastic activity of compound 5.

In view of the inhibition results found for compounds 3 and 5, we sought to understand the molecular factors involved in these results of antineoplastic activity from molecular docking studies involving the tubulin protein, a pharmacological target widely recognized for its role in anticancer therapies[23]. Furthermore, the tubulin protein has often been suggested as a possible drug target activity for antineoplázica oxadiazólicos derivatives[30,36]. The binding free energy values are summarized in table 2.

As shown in table 2, the expected binding energy value for compound 3 was -6.72 Kcal/mol. However, a lower binding energy value (-8.11 Kcal/mol) was obtained for compound 5. Both molecules showed higher energy values than colchicine, a drug widely known for its anti-tubulin effects, however, these values were not far removed from that found for this standard drug.

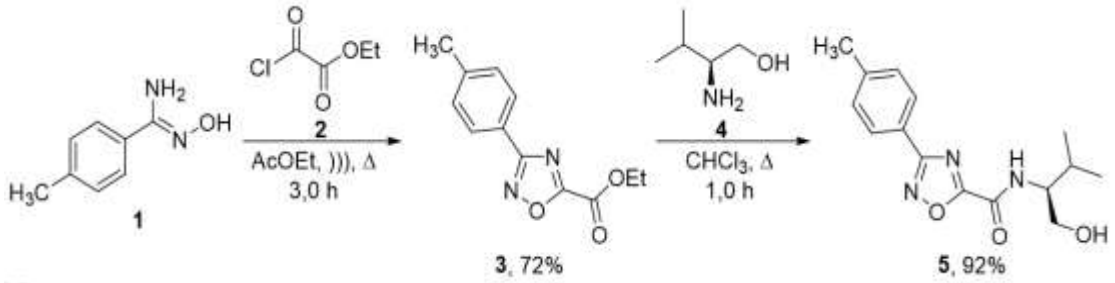


Figure 2: Synthesis of compound (*S*)-*N*-(1-hydroxy-3-methylbutan-2-yl)-3-(p-toluyl)-1,2,4-oxadiazole-5-carboxamide (5).

Table 1
Percentage of inhibition in a single concentration (25 µg / mL) of the cell growth of the samples in three tumor lines and their standard deviation from the mean.

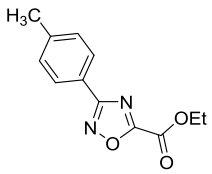
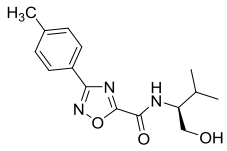
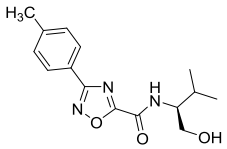
Compound		HL60(%)	HCT 119(%)	MCF-7(%)
	**3**	0,0	7,79 (±2,08)	0,0
	**5**	25,39 (±7,9)	28,30 (±15,2)	31,16 (±2,75)

Table 2
Free binding energies of compounds 3 and 5 and colchicine against tubulin.

Compound	Free binding energies ΔG (kcal/mol)
3	-6,72
5	-8,11
Colchicina	-9,90

Table 3
Pharmacokinetic, toxicological and chemical properties *in silico* of compound 5.

Product	Properties									
	cLogP	**MM**	**nHBD**	**nHBA**	**Lipinski**	**GI**	**CT**	**DL**	**LL**	**DS**
	2,08	289,33	2	5	0	High	low Risk	3,32	Yes	0,85

cLogP: Log $P_{o/w}$ consensual; MM: Molecular mass; nHBD: Number of hydrogen bonding donors; nHBA: Number of hydrogen bond acceptors; Lipinski: Number of violations of Lipinski's rule; GI: Gastrointestinal absorption; CT: chronic toxicity; DL: *Druglikeness*; LL: *Leadlikeness*; DS: *Drug Score*

In addition to the molecular docking studies, other *in silico* studies were performed with compound 5 in order to identify promising characteristics that encourage the development of more advanced studies involving this molecule and its possible derivatives. For this, the SwissADME and Osiris Property Explorer platforms (free web-based programs) were used to obtain the pharmacokinetic, toxicological and chemical characteristics of this compound. These results are summarized in table 3.

The *in silico* analyzes showed that compound 5 meets all the requirements addressed by the "Rule of 5" developed by Lipinski et al[25] indicating a high probability of good bioavailability of this compound after its oral administration. This indication is also supported by the result of the estimate of good gastrointestinal absorption (GI) assessed by the method described by Daina and Zoete[9].

In addition to the good expectation of oral bioavailability, compound 5 also had a low risk of presenting chronic toxicity and especially a high probability of becoming a drug in the future as indicated by the values of Druglikeness (DL), Leadlikeness (LL) and Drug Score (DS).

Together, these results highlight the proposed methodology for obtaining amino-oxadiazoles, which contributed to improving the antineoplastic activity of compound 3 providing the compounds with good pharmacokinetic, toxicological and chemical characteristics.

Discussion

The yield values and reaction time for compound 3 were 72% and 3.0 h, values similar to the value described by Mayer et al.[27] However, in the protocol of Mayer et al[27] and Voronova et al[47], toxic reagents such as triethylamine, *N*, *N*-diisopropylethylamine (DIPEA) and acetonitrile were used. The literature does not describe the synthesis of compound 5.

After analyzing the infrared spectrum of compound 5, a band at 3234 cm^{-1} was observed relating to the N-H stretch of amide bond; this being an important band showing the formation of this compound. 3377 cm^{-1} broad band was observed referring to stretching of the bonding O-H, alcohol characteristic[34]. Moreover, it was possible to observe bands at 3053 cm^{-1} relating to the stretching of the C-H sp^2 carbon and 2961 cm^{-1} related to the stretching of the C-H sp^3 carbon. 1684 cm^{-1} intense band was assigned to the amide carbonyl group and 1684 cm^{-1} intense band was assigned to the stretch of the C=N bond oxadiazole ring.

In the ^1H NMR spectrum of compound 5, a signal was observed at 7.97 ppm and another at 7.18, both with multiplicity of the double and integral type 2 which were attributed to the aromatic hydrogens. The signal at 2.42 ppm singlet multiplicity, integral type 3 has been attributed to the hydrogens of the CH_3 group attached to the aromatic ring. The other signals confirm the incorporation of the (*S*)-(+)-2-amino-3-methyl-1-butanol group, for example, the signals at 1.02, 1.06 ppm (CH_3 hydrogens with multiplicity of doublet type and integrals equal to 3), 2.06 ppm (CH hydrogen with multiplicity of octet type and integral 1), 3.85 (CH_2 hydrogens with multiplicity of doublet type and integral 2) and between 4.00-3.93 (hydrogen multiplet CH).

In the ^{13}C NMR spectrum of compound 5, thirteen signals were observed, this value corresponding to the number of chemically different carbons present in the structure of this compound. The signal observed at 168.5 ppm was assigned to the amide carbonyl carbon signals at 168.7 and 153.5 ppm and was assigned to the carbons of the oxadiazole ring. Additionally, Asgari, Memarian and Sabzyan[3] reported that the 1,2,4-oxadiazolic ring carbons generally present a chemical shift between 150 and 180 ppm. The other signals present in the ^{13}C NMR spectra confirm the structure of the compound.

After obtaining and characterizing compounds 3 and 5, we sought to evaluate their anti-cancer properties, since recent studies have pointed out the inhibitory potential of different 1,2,4-oxadiazoles and their derivatives against cancer cell lines[17].

As a result, a low inhibition of compound 3 was compared to the tested strains, inhibiting only the HCT 116 cell line. In contrast, it was possible to observe that the insertion of the amide portion in compound 3 had a positive impact on the antineoplastic activity of compound 5 with higher inhibition values being observed in relation to its precursor for all tested cells, reaching 31.16% for the strain MCF-7.

Given this result, it is possible to verify that compounds 3 and 5 showing little antineoplastic activity with the substances classified as having no activity according to Souza[41].

It is possible to notice that the results of the antineoplastic evaluation of compounds 3 and 5 are in agreement with the values of free energy of binding predicted in the study of molecular docking for these compounds in the active site of the tubulin protein, since the compound 5 presented a value for less energy than that found for compound 3, corroborating the result of antineoplastic activity *in vitro*.

Colchicine has a specific interaction site on the tubulin protein structure located at the interface between the α and β subunits of the heterodimer[37]. The analysis of the anchorage mode of compound 5 shows its ability to position itself at this interface interacting with amino acids belonging to both the α subunit and the β subunit (Figure 3). Once at the active site, the methyl group attached to the aromatic ring of compound 5 interacts with the VAL:181 residue present in the α subunit, while the π-electron cloud of the oxadiazolic nucleus establishes π-σ type interactions with LEU:255, π - alkyl with ALA:250 and amide- π stacking with LYS:254, all in the β subunit. The formation of hydrogen bonds with the residues of VAL:238, CYS:241 and ASN:258 is also noted. Additionally, other interactions are established between compound 5 and the colchicine site of tubulin protein as shown in figure 3.

The low value of free binding energy of compound 5 is indicative of its possible interaction with the active site of colchicine in the tubulin protein, since the lower is the free binding energy, the greater is the stability of the ligand-receptor complex and therefore, interaction will be more favored[12]. These results converge with the data observed in other studies that indicate the interaction of tubulin as a probable mechanism of action for the antineoplastic activity of oxadiazolic derivatives[1,17,22].

Additionally, other computational studies were performed for compound 5 to identify promising characteristics that encourage the development of more advanced studies involving this compound and its derivatives. The first important point shown in table 3 is the lipophilicity attributed to compound 5 expressed by the cLogP value. The lipophilicity is important because a drug needs to be well absorbed and permeates the body barriers to reach its site of action.

According to Barreiro and Fraga[4], there is an ideal lipophilicity range for a drug which has molecules with LogP values between 1 and 3. Thus, it is possible to note that compound 5 is within this ideal lipophilicity range, therefore, good pharmacokinetic characteristics are expected for this compound.

This result is reinforced when we analyze the physical-chemical characteristics of compound 5 in table 3 from the "Rule of 5" developed by Lipinski et al[25] when analyzing 2,245 drugs that showed good standards of oral bioavailability and are widely disseminated and used routinely in new drug discovery protocols. This rule establishes that a molecule will present a good standard of oral bioavailability when it meets a set of physical-chemical parameters which are: molecular mass (MM) less than 500 Daltons, partition coefficient (cLogP) less than 5 maximum of five H-bond donor groups (nHBD) and a maximum of ten H-bond acceptor groups (nHBA)[37].

The results shown in table 3 corroborate what was pointed out in the discussion about the lipophilicity of compound 5, since this compound meets all these requirements indicating an excellent potential for oral bioavailability.

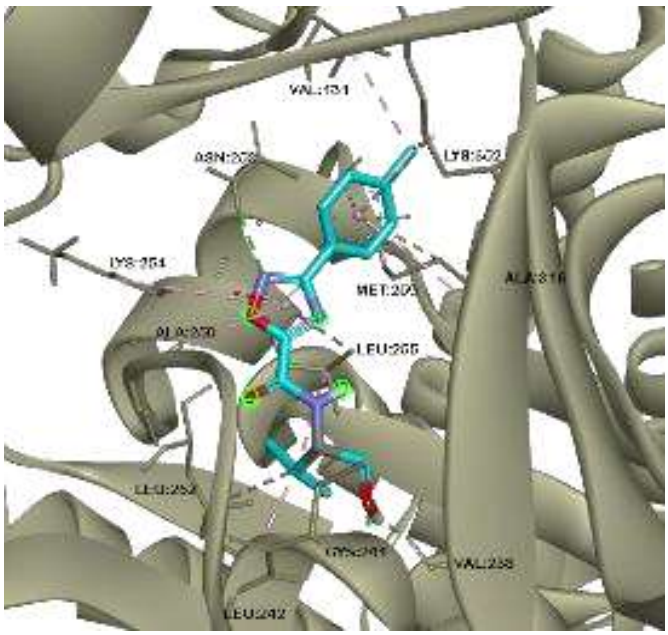


Figure 3: Interactions between compound 5 and colchicine site on the tubulin protein.

This is a result of great importance, since this route of administration brings unique benefits such as convenience, low cost, possibility of self-administration, greater adherence to treatment and lower risk of triggering systemic infections in the user[16].

The expectation of good oral bioavailability is reinforced by the high gastrointestinal absorption potential (Table 3) which is information obtained by the SwissADME platform[27]. Table 3 also shows the probability of this compound showing chronic toxicity (CT), an analysis that expresses the ability of this compound to present mutagenicity, tumorogenicity, irritability and interference in human reproduction. This survey was carried out using Osiris Preperty Explorer, classifying compound 5 as a chemical structure with a low risk of chronic toxicity, similar to what is noted for the vast majority of drugs analyzed by this platform.

The good results of lipophilicity, oral bioavailability and chronic toxicity pointed out in computational studies highlight compound 5 as a promising molecule for the development of new drugs, since 80% of the molecules start research to become new drugs and do not reach clinical studies, 50% of them have failure due to their unfavorable pharmacokinetic and toxicological properties[29]. This shows that in addition to a good pharmacodynamic action, there must be a satisfactory balance between pharmacokinetic and toxicological characteristics in order to sustain the use of a molecule.

Finally, table 3 shows the computational results that express the probability that a molecule will become a new drug based on its physical-chemical and biological characteristics as well as on its similarity with other molecules already on the market which are the Druglikeness data, Drug Score (calculated by Osiris Property Explorer) and Leadlikeness (calculated by SwissADME).

The Leadlikeness expresses the result of a chemical-physical filter used in the development of new drugs that indicates whether a particular molecule has characteristics "leading to similar compounds"[10]. Thus, it was shown that compound 5 has similar features to the compounds that lead the drug development process. In turn, *Druglikeness* quantitatively assesses the similarity of the tested compound with a list created from the fragmentation of 3,300 commercial drugs and 15,000 non-medicated chemicals present in the Fluka® catalog, resulting in a complete list of all available fragments[46].

According to the Osriris platform, the ideal is for a candidate to present Druglikeness values greater than 0 indicating its similarity with commercial drugs. Thus, it was possible to observe in table 3 a result well above 0 for compound 5 indicating the chemical similarity of this compound with the commercial drugs used in the elaboration of the Osiris platform.

Meanwhile, the Drug Score is calculated from physicochemical and biological factors expressing the probability that a molecule will become a drug in the future.

According to the established criteria, the closer to 1 (one) the drug score result is, the greater is the theoretical probability that this molecule will become a good drug. Compound 5 obtained a score of 0.85 indicating a harmony between its molecular characteristics and indicating a high probability of becoming a good drug.

Several factors have pointed to the need for new antineoplastic agents, especially the large number of cancer cases that are being noticed daily, many of them with serious repercussions leading many patients to death[24].

In addition, many problems are noted in anti-cancer therapies due to their high toxicity and non-specific action, not differentiating the affected cells from healthy ones, in addition to the frequent cases of tumor cell resistance to therapeutic agents which have often negatively impacted the well-being of the patient being treated.

Given this need, one of the most important steps in the discovery of new drugs is the constant search for new prototypes from planned molecular changes, thus contributing to the development of new compounds that may provide better risk/benefit patters[26].

Thus, these results mark the first step in the development of new drugs belonging to the class of amino oxadiazoles, providing information that will be useful in conducting future studies, since the results presented here highlight compound 5 as a promising candidate for the process of optimization with a good chance of becoming a good drug in the future.

Conclusion

(*S*)-*N*-(1-hydroxy-3-methylbutan-2-yl)-3-(*p*-toluyl)-1,2,4-oxadiazole-5-carboxamide (5), a new 1,2, 4-oxadiazole, was prepared in excellent yield (92%) and in quick reaction time (1.0 h). Compound 5 showed better antineoplastic activity when compared to its precursor, that is, compound 3. This result was corroborated by molecular docking studies which also pointed to the interaction with tubulin as a probable mechanism of action for its antineoplastic activity.

The other *in silico* study analyzes highlighted compound 5, pointing out excellent pharmacokinetic, toxicological and chemical characteristics, theoretically supporting a good probability of its oral absorption, presenting a low risk of developing chronic toxicity effects and expressing a high probability of becoming a good drug in the future.

These results mark the first step in the process of discovering and optimization of novel drugs of this class of compounds providing important information for conducting future studies involving such derivatives.

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