Research Article

Novel Doxorubicin Derivatives for Reduced Toxicity: An In Silico Study

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ABSTRACT

Doxorubicin is a chemotherapy drug, classified as an anthracycline antibiotic, and has been used as a form of treatment since the 1960s. It is used to treat various cancers, including blood cancers, such as leukemia and lymphoma, as well as solid tumors, such as breast, bladder, lung, and ovarian cancer. Doxorubicin can inhibit the growth and kill cancer cells at any stage, making it one of the most effective chemotherapy drugs. The majority of chemotherapy drugs have severe side effects, as their cytotoxic nature forces them to target healthy cells in addition to cancerous cells. Doxorubicin, in particular, is one of the most toxic chemotherapy drugs and can lead to life-threatening cardiac conditions. Doxorubicin's toxicity limits its potential and effectiveness, as doses are administered cautiously, with the maximum lifetime cumulative dose being 550 milligrams per square meter. This study aims to reduce the toxicity of doxorubicin by developing five alternative derivatives that lower toxicity levels while maintaining the function of the original compound. All five derivatives showed improvement in toxicity, with the most significant changes in derivative 4. Derivative 4 completely removed the risk of neurotoxicity and cytotoxicity and reduced the toxicity of four groups by at least 26%, some up to 33%. Since the backbone structure remained unchanged, the mechanism of action is likely the same. While these derivatives still need to be further explored and tested through clinical trials, they are a promising alternative for safer and less harmful versions of doxorubicin.

Keywords: Apoptosis; Cancer; Free Radicals; Toxicity; Oxidation

INTRODUCTION

In the human body, cells rapidly divide through the process of mitosis, allowing for growth. Cancer occurs when the process of cell division results in a mutation that causes cells to multiply uncontrollably, forming a tumor. Malignant tumors have the ability to invade surrounding tissues and spread to other parts of the body. Doxorubicin is a chemotherapy drug that works to kill cancer cells and hinder the reproduction of those cells.

The primary mechanism of action involves its ability to bind to DNA by inserting itself between DNA base pairs through the intercalation of the anthraquinone ring, thereby disrupting the structure and inhibiting the enzyme topoisomerase II. During the process of DNA replication, topoisomerase II relieves supercoiling and tangles by cutting and reattaching strands to create coherent DNA strands. When doxorubicin interferes

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with this mechanism, strands undergo supercoiling and torsional strain, resulting in DNA damage and apoptosis. Additionally, doxorubicin can generate free radicals, which are unstable molecules with an unpaired electron that cause oxidative DNA damage. When doxorubicin is reduced by enzymes, it forms a semiquinone free radical, which reacts with oxygen to produce superoxide and hydrogen peroxide, both of which are reactive oxygen species. These free radicals interact with iron from the body's cells, and doxorubicin binds to the iron to create a doxorubicin-iron complex. This complex works to form additional free radicals, such as hydroxyl radicals, that may have stronger effects on the DNA. Free radicals cause cell death by attacking the DNA and inflicting breakage, directly damaging DNA bases and the sugar-phosphate backbone. Oxidative stress can prompt programmed cell death in cancer cells.

Lipinski's Rule of Five is a criterion made to assess drug properties such as solubility, bioavailability, and efficacy. The criterion involves molecular weight, hydrogen bond donors, hydrogen bond acceptors, and lipophilicity. It states that the molecular weight must be less than 500 g/mol, the molecule must have less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, and a logP value less than 5, with only one violation allowed. Doxorubicin has a molecular weight of 543.5 g/mol, 6 hydrogen bond donors, 12 hydrogen bond acceptors, and a lipophilicity of 1.27 (1). Besides molecular weight, which exceeds 500 g/mol, and hydrogen bond acceptors, which exceed 10, doxorubicin meets the criteria; however, because it is an antibiotic, it is considered an exception, as they often violate these rules. Lipophilicity indicates a molecule's preference to dissolve in the organic solvent octanol compared to water, and typically ranges from -3 to 7, with -3 being very hydrophilic and 7 being very lipophilic. A logP value of 1.27 indicates that it is moderately soluble, with 2% solubility in water, and is slightly more soluble in lipid environments. It falls within the ideal range for lipophilicity as there is a balance between water solubility and membrane permeability. Doxorubicin is administered through an intravenous injection, allowing it to directly enter the bloodstream and cross cell membranes to reach DNA.

Doxorubicin is considered one of the most toxic chemotherapy drugs, having a predicted toxicity class of 3 on the scale of 1 through 6, with 1 being the most harmful and 6 being the least. It has 10 active toxicity areas, the most prominent being immunotoxicity, with a 99% probability, and mutagenicity, with a 98% probability. These are followed by high probability of cytotoxicity, respiratory toxicity, nephrotoxicity, neurotoxicity, and clinical toxicity. Furthermore, there is significant potential for cardiotoxicity, as 11% of patients experience acute cardiac toxicity, and 26% of patients develop arrhythmias, including sinus tachycardia, premature atrial and ventricular contractions, and supraventricular tachycardia. Congestive heart failure and cardiomyopathy can also occur, resulting in a 50% mortality rate after 1 year of administration (2). There are many reasons for its toxicity, including its inability to distinguish between cancer cells and normal cells. Specifically for doxorubicin, its use of free radicals facilitates fatal cardiotoxicity. Doxorubicin undergoes redox cycling and forms reactive oxygen species, which attack cardiomyocytes in the heart. The heart responds to this stress by releasing hormones such as brain natriuretic peptides and atrial natriuretic peptides, which provoke cardiac hypertrophy. Additionally, the production of cytokines and cytotoxic T lymphocytes encourages the presence of natural killer cells. While natural killer cells are white blood cells that fight against cancer, they put tremendous strain on the heart while doing so (3).

Neurotoxicity is also a major component of doxorubicin's toxicity profile. Although it is unable to cross the blood-brain barrier, it indirectly causes harm by stimulating the production of a cytokine signaling protein called the tumor necrosis factor alpha. This protein works to regulate inflammation and immune responses and can provoke microglial cells in the brain to generate inflammatory cytokines. The enzyme inducible nitric oxide synthase, which produces nitric oxide, is released and increases levels of reactive nitrogen species. This forces the surrounding proteins to undergo nitration and create additional reactive oxygen species, the same free radicals that are used for doxorubicin to prompt cancer cell death. As these reactive oxygen species reach the brain and enter the mitochondria's permeability transition pore, the release of the cytochrome c protein leads to apoptosis. This results in cognitive impairment, the damaging of neurogenesis, where new neurons are formed, and dysregulation of neurotransmitter production (3, 4).

Additional sources of toxicity include damage to the liver and kidneys. 40% of patients receiving treatment experience impairment of the liver, as the liver absorbs the majority of the drug and metabolizes it. This process causes overproduction of reactive

oxygen species, which damages DNA, induces lipid peroxidation, decreases vitamin E and adenosine triphosphate (ATP) levels, and reduces glutathione. In the kidney, nephrotoxicity occurs when doxorubicin causes nephropathy and proteinuria by damaging the glomerular podocytes, specialized cells that filter blood to form urine. When unwanted proteins come in contact with exposed renal tissue, the nephron structure is modified, and glomerulosclerosis occurs. Because the kidney does not easily regenerate, the glomerulus is often permanently damaged, causing further injuries such as glomerular lesions, inflammation, and tubular dilation (3). Toxicity remains the primary limitation for doxorubicin, as its maximum safe dosage constrains its effectiveness.

The primary objective of this study is to reduce the overall toxicity of doxorubicin while maintaining its efficacy. We altered the structure of doxorubicin to create five novel derivatives, which all significantly reduced the toxicity, with the most substantial reduction in neurotoxicity. In this process, we identified five alternate versions of doxorubicin that have decreased toxicity and would likely perform better as a higher dosage could be given with fewer harmful side effects. Doxorubicin and five novel derivatives are modeled in a 2D plane shown below.

METHODS AND MATERIALS

PubChem was used to determine the original structure and characteristics of doxorubicin. To computationally model new derivatives of doxorubicin, we used the "draw structure" feature on PubChem. This software is the PubChem Sketcher, a JavaScript editor developed by the National Institutes of Health. After alterations were made, the simplified molecular input line entry system (SMILES) of the molecule was saved and documented. SMILES is a chemical structure line notation used for representing molecules. Each derivative molecule was entered into ProTox 3.0, using the Tox Prediction feature to evaluate the changes in toxicity. The Tox Prediction indicates the predicted toxicity class, information about its molecular weight, hydrogen bond acceptors, hydrogen bond donors, rotatable bonds, logP value, active and inactive toxicity groups, and its predicted probability. It should be noted that ProTox 3.0 has certain limitations in its accuracy, and predictions should be further validated with lab testing. We used Canva to format and arrange the figures.

RESULTS AND DISCUSSION

The five derivatives of doxorubicin all show an improvement in toxicity. Some completely remove the risk of certain toxicities by making the group inactive, while others reduce the probability of occurrence. Two or fewer alterations were made for each new molecule, and the backbone structure remained untouched to preserve its original function and fundamental shape. The backbone, which contains anthraquinone rings with two ketone groups and two hydroxyl groups, is the part of the structure that intercalates between DNA base pairs. Within the molecule, group A refers to an amino sugar, specifically a daunosamine. Group B is known as the anchor region and contains an alcohol group and a ketone group. The main cause of toxicity from doxorubicin involves aggressive free radicals interfering with primary mechanisms in various organs and systems. Thus, the reduction of free radicals directly reduces overall toxicity. However, it should be noted that computational predictions may not fully translate into biological systems.

Derivative 1: replacing oxygen with nitrogen

Derivative 1 was created by changing the oxygen in group A to nitrogen with a bonded hydrogen (Figure 1). This alteration was chosen as excess oxygen atoms were causing additional harm. This resulted in a reduction of mutagenicity by 43.87%, cytotoxicity by 36.17%, nephrotoxicity by 15.00%, and neurotoxicity by 14.86%. It also slightly reduced cardiotoxicity by 10.93%, respiratory toxicity by 6.59%, and clinical toxicity by 4.70% (Figure 2). Oxygen is a source of free radicals, so when the atom is removed, it reduces the number of free radicals, specifically reactive oxygen species, that are being produced. Oxygen has two lone pairs, whereas nitrogen only has one lone pair, so oxygen has a higher electron density and increases the chance that an electron can become unpaired and form a free radical. Oxygen is also more likely to generate a free radical because it has a higher electronegativity, suggesting a higher affinity for electrons, which can lead to uneven electron distributions during bond breaking. Nitrogen reduces the chances of producing free radicals, and even if it does produce radicals, reactive nitrogen species are generally more stable and less destructive.

Derivative 2: replacing oxygen with sulfur

Derivative 2 was created by replacing the oxygen in group A with sulfur (Figure 1). This resulted in a

reduction of nephrotoxicity by 21.25%, mutagenicity by 20.40%, neurotoxicity by 17.56%, cytotoxicity by 14.89%, cardiotoxicity by 10.93%, clinical toxicity by 9.52%, and respiratory toxicity by 3.20% (Figure 2). Sulfur and oxygen are in the same group and have the same number of valence electrons and lone pairs. However, sulfur has a lower electronegativity and a larger atomic radius compared to oxygen. This makes it harder for sulfur to form free radicals, as a larger atomic radius and size allow more space for radicals to move around and therefore are more stable and polarizable. Sulfur-based free radicals take longer to form and are less reactive.

Derivative 3: removing group A and group B

Derivative 3 involves removing both group A (daunosamine) and group B (anchor region) (Figure 1). This alteration was chosen to isolate the backbone and remove unnecessary components of the structure. In this process, we remove 6 oxygen atoms, so the possibility of forming reactive oxygen species is significantly decreased. Most notably, the possibility of neurotoxicity

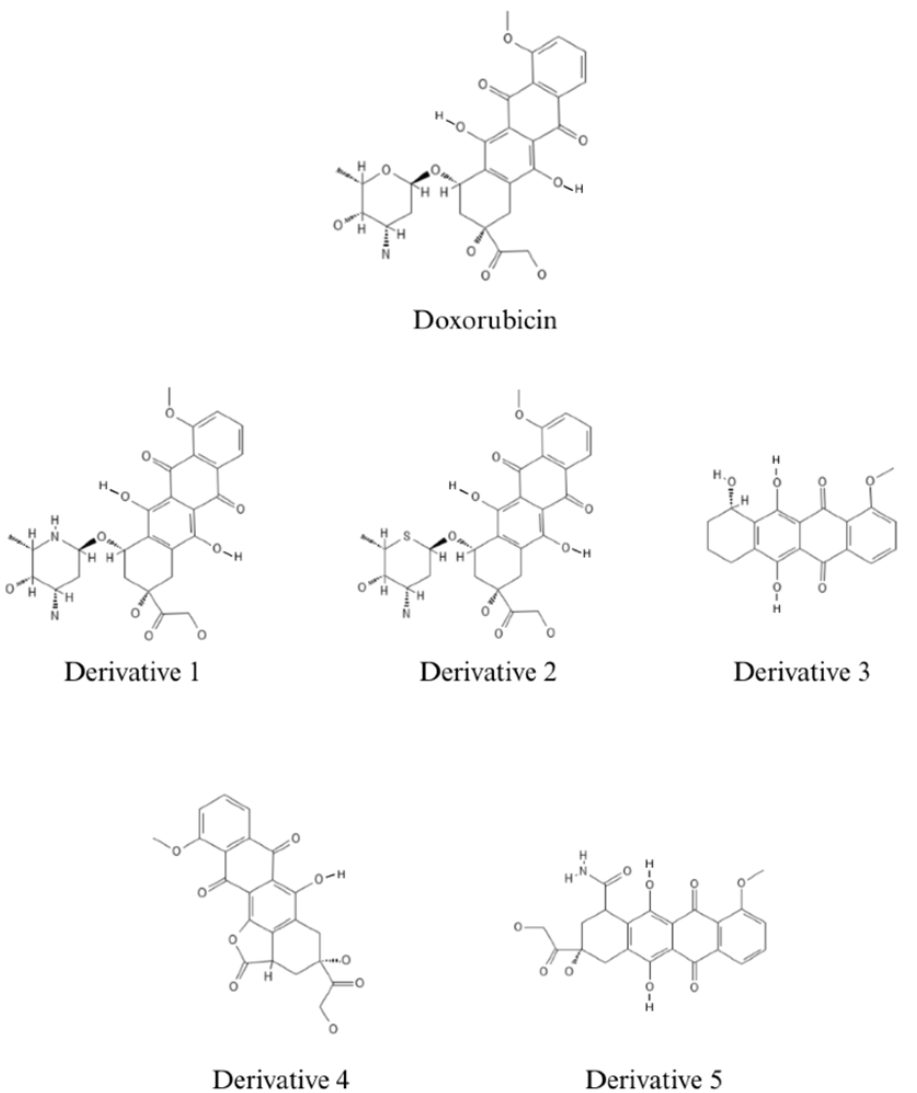

Figure 1. The molecular structure of doxorubicin and the molecular structure of the five novel derivatives of doxorubicin. The molecular structure was computationally altered using the JavaScript PubChem Sketcher on PubChem. PubChem is a chemical database created by the National Center for Biotechnology Information at the U.S. National Library of Medicine.

and cytotoxicity is completely eliminated as those groups become inactive. Nephrotoxicity was reduced by 22.50%, mutagenicity was reduced by 22.44%, respiratory toxicity was reduced by 21.97%, clinical toxicity was reduced by 17.85%, and cardiotoxicity was reduced by 4.68% (Figure 2).

Derivative 4: removing group A and replacing it with a carboxyl group

Derivative 4 was produced by removing group A and replacing it with a carboxyl group (COOH) (Figure 1). Derivative 4 produced the best results, improving toxicity by making neurotoxicity and cytotoxicity inactive. Additionally, it reduced nephrotoxicity by 33.75%, mutagenicity by 31.63%, respiratory toxicity by 27.47%, clinical toxicity by 26.19%, and cardiotoxicity by 12.50% (Figure 2). When the carboxyl group is attached to the remaining hydrogen left from group A, it combines with the hydrogen to form water (H_2O), which dissolves and results in only carbon and oxygen remaining. Due to the proximity of the carboxylic acid and alcohol, rapid dehydration occurs, resulting in the formation of the ring. Despite adding oxygen atoms, this new ring is more stable than the previous one because of aromaticity. Aromaticity is the property that involves delocalized electrons in conjugated cyclic structures to increase a molecule's stability. Conjugated cyclic structures refer to rings that alternate between single and double bonds to allow pi electrons to delocalize and travel across the ring. This increases chemical stability, as the delocalization of electrons lowers the overall energy, maintains a stable electron shell, and decreases reactivity.

Doxorubicin

Target	Prediction	Probability
Hepatotoxicity	Inactive	0.86
Neurotoxicity	Active	0.74
Nephrotoxicity	Active	0.80
Respiratory toxicity	Active	0.91
Cardiotoxicity	Active	0.64
Carcinogenicity	Inactive	0.90
Immunotoxicity	Active	0.99
Mutagenicity	Active	0.98
Cytotoxicity	Active	0.94
BBB-barrier	Inactive	1.0
Ecotoxicity	Inactive	0.58
Clinical toxicity	Active	0.84

Derivative 1

Prediction	Probability
Inactive	0.85
Active	0.63
Active	0.88
Active	0.85
Active	0.51
Inactive	0.8
Active	0.99
Active	0.55
Active	0.60
Inactive	0.86
Inactive	0.63
Active	0.80

Derivative 2

Prediction	Probability
Inactive	0.80
Active	0.61
Active	0.63
Active	0.88
Active	0.57
Inactive	0.85
Active	0.99
Active	0.78
Active	0.80
Inactive	0.84
Inactive	0.58
Active	0.76

Derivative 3

Target	Prediction	Probability
Hepatotoxicity	Inactive	0.73
Neurotoxicity	Inactive	0.81
Nephrotoxicity	Active	0.62
Respiratory toxicity	Active	0.71
Cardiotoxicity	Active	0.61
Carcinogenicity	Inactive	0.70
Immunotoxicity	Active	0.99
Mutagenicity	Active	0.76
Cytotoxicity	Inactive	0.78
BBB-barrier	Inactive	0.57
Ecotoxicity	Inactive	0.70
Clinical toxicity	Active	0.69

Derivative 4

Prediction	Probability
Inactive	0.84
Inactive	0.84
Active	0.53
Active	0.66
Active	0.56
Inactive	0.73
Active	0.99
Active	0.67
Inactive	0.77
Inactive	0.58
Inactive	0.69
Active	0.62

Derivative 5

Prediction	Probability
Inactive	0.80
Inactive	0.58
Active	0.61
Active	0.65
Active	0.53
Inactive	0.68
Active	0.99
Active	0.58
Inactive	0.56
Inactive	0.53
Inactive	0.66
Active	0.73

Figure 2. The original toxicity predictions of doxorubicin compared to the predicted toxicities of the five novel derivatives of doxorubicin generated by ProTox 3.0. Protox 3.0 is a computational tool that uses machine learning techniques and toxicological datasets to predict the toxicities of chemical compounds.

Derivative 5: removing group A and replacing it with an amide group

Derivative 5 was created by removing group A and replacing it with the amide, $CONH_2$ (Figure 1). Derivative 5 made neurotoxicity and cytotoxicity inactive. The probability of mutagenicity was reduced by 40.81%, the probability of respiratory toxicity was reduced by 28.57%, the probability of nephrotoxicity was reduced by 17.56%, the probability of cardiotoxicity was reduced by 17.18%, and the probability of clinical toxicity was reduced by 13.09% (Figure 2). An amide has one double-bonded oxygen, which is considered a stable oxygen atom and contributes to the stability of the entire functional group. A more stable group is less likely to undergo radical formation. Even if a free radical forms, it will be less reactive due to electron delocalization. Specifically, moving a lone pair from oxygen to nitrogen lowers the overall energy of molecules and is thus less likely to be unstable and form a free radical. This is a better alternative to the original molecule, as group A does not have the space for delocalized electrons and cannot undergo this process.

It is noteworthy that the removal of the daunosamine ring in group A for derivatives 3, 4, and 5 may result in minor changes in the potency of doxorubicin. The daunosamine ring is responsible for releasing an abundance of free radicals and contributing to neuroinflammation; therefore, removing it deactivates neurotoxicity. Conversely, it enhances doxorubicin's ability to bind to DNA, as the sugars in daunosamine provide stability through groove binding during intercalation, and its absence may weaken the inhibition of topoisomerase II. The anthraquinone ring backbone also facilitates DNA intercalation and cell death, and is the primary mechanism of this process. Given that the anthraquinone structure remains unaltered, only a slight reduction in efficacy would be expected due to changes in binding stability.

Immunotoxicity does not change in any of the derivatives and remains active with a 99% probability. This is because these derivatives focused on the reduction of free radicals to improve toxicity, and the general reduction of free radicals improved toxicity for systems that were being directly affected by oxidative stress. Although the mechanism of immunotoxicity from doxorubicin involves free radicals, it also involves immune cell damage during DNA intercalation, the triggering of immunosuppressive genes, and the disruption of cytokine signaling pathways to the immune system. This suggests that oxidative stress is not the main source of immunotoxicity and explains the lack of improvement compared to other toxicities. In order to reduce immunotoxicity, we would need to target specific mechanisms that directly affect the immune system.

The lipophilic values vary across the derivatives, with the original logP of doxorubicin being 1.27. The logP values of derivatives 1 through 5 are 0.99, 2.07, 4.27, 1.71, and 2.44, respectively. The optimal range for an intravenously injected drug is between 1 and 3, as it requires both water solubility to circulate and enter the bloodstream, as well as lipophilicity to cross cell membranes. Besides derivatives 1 and 3, which do not meet this range, the rest of the derivatives are in the ideal range on the lipophilicity scale. Nonetheless, they all meet Lipinski's criteria for having a logP value less than 5.

CONCLUSION

The objective of this study was to reduce the overall toxicity of doxorubicin. We did this by altering the structure of doxorubicin through computational modeling to create novel derivatives that reduced toxicity by decreasing the production of free radicals. This diminished levels of oxidative stress and created safer versions of doxorubicin. The anthraquinone rings serving as the backbone of the structure that allow for DNA intercalation remained intact, suggesting that the antibiotic's primary function was preserved.

All five derivatives show a significant improvement in toxicity, and we propose that derivative 4 shows the most potential for a safer alternative to doxorubicin. Derivative 4 eliminated the risk of neurotoxicity and cytotoxicity, both of which were major concerns for adverse effects. It reduced the probability of nephrotoxicity by 33.75%, mutagenicity by 31.63%, respiratory toxicity by 27.47%, clinical toxicity by 26.19%, and cardiotoxicity by 12.5%. Furthermore, derivative 4 had a logP value closest to the logP of doxorubicin, implying that it would behave most similarly to the original drug. These results demonstrate that complications and side effects in patients receiving doxorubicin treatment would be minimized with our modifications. With additional research on binding affinities and toxicity analysis, this study can begin further investigation in clinical testing.

CONFLICTS OF INTEREST

There are no conflicts of interest regarding the publication of this paper.

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