Derivatives of doxorubicin for treatment of breast cancer

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ABSTRACT

Breast cancer is a type of cancer that usually appears within the inner lining of the milk ducts or lobules. Treatment will include surgery, drugs (hormone therapy and chemotherapy), and radiation or combination thereof. This study presents eight drug derivatives of doxorubicin for treatment of breast cancer. These compounds are derived through substituent replacement onto the molecular scaffold of doxorubicin. Pharmaceutical properties of these compounds (inclusive of doxorubicin) such as 1-octanol/water partition Log P varied from 0.567 to 4.137, rotatable bonds varied 5 to 12, polar surface area from 195.084 Å² to 206.08 Å², and water solubility 0.00873mg/L to 390mg/L. The number of oxygen and nitrogen atoms (hydrogen bond acceptors) remained constant at 12 for all compounds. Although violations of the Rule of five remained constant at three for all compounds, the variation of Log P and water solubility offers considerable and beneficial potential of this group of anticancer agents to enhance the anticancer activity of these anthracycline antibiotics. The variation of molecular substituents covalently bonded within the doxorubicin structure clearly allow advantageous analogs that may be effective pharmaceuticals for the clinical treatment of breast cancer.

INTRODUCTION

Breast cancer is a type of cancer initially appearing in the inner lining of the milk ducts or lobules. There are various sorts of breast cancer that will have varying levels of proliferation, aggressiveness, and genetic constitution. Survival rate varies depending on these factors. The 10-year disease-free survival rate can vary from 10% to 98%. Treatment includes surgery, drugs (hormone therapy and chemotherapy), radiation or some combination. Breast cancer is the second most common type of cancer after lung cancer. Doxorubicin is an anthracycline antibiotic that will inhibit the growth of bacteria but is not usually applied in clinical treatment due to the considerable level of toxicity to humans[1].

The anthracycline antibiotic doxorubicin is applied in the clinical treatment of many types of cancers such as breast cancer, hematological malignancies, various carcinomas, and soft tissue sarcomas[1-3]. Doxorubicin itself is light sensitive and is generally administered intravenously[1-3]. The anthracyclines as a class of drugs constitute some of the most effective anticancer agents having effectiveness against more types of cancers than any other class of chemotherapeutic agents[4,5].

Unfortunately they express a clinically problematic cardiotoxicity that can increase in threat as patient survival rate increases[4,6]. However the use of doxorubicin in combination with paclitaxel for the treatment of
metastatic breast cancer, has demonstrated beneficial outcome at over 90% response rate. The problematic appearance of neutropenia and mucositis does complicate treatment regimen and outcome results. Descriptive of anthracycline antibiotic use, is the occurrence of congestive heart failure that is found in 20% of the patients. Also the occurrence of mucositis and neutropenia can place dose limitations on the use of doxorubicin, even in combination with paclitaxel. However the use of paclitaxel in combination with doxorubicin is corroborated as an effective antineoplastic treatment for metastatic breast cancer.

Other studies have shown that the application of doxorubicin in liposomal form may reduce toxicity usually associated with anthracyclines. Particularly the use of liposomal doxorubicin with platinum agent may benefit therapy of advanced malignant epithelial ovarian carcinoma. Encouraging preliminary results have been observed in the treatment of advanced solid tumors by pegylated liposomal doxorubicin with paclitaxel. In those studies there was a reduced appearance of neutropenia and cardiotoxicity, forming a regimen of acceptable toxicity. In other case studies the use of pegylated liposomal doxorubicin with platinum also demonstrated decreased neurotoxicity but encouraging outcome for ovarian cancer patients.

Clearly the investigation of novel applications and designs of anthracycline antibiotics will benefit therapeutic approaches to the treatment of cancer. This work presents the application of in silico optimization with pattern recognition analysis to optimize structural modifications to improve pharmaceutical activity of already proven antineoplastic compounds.

EXPERIMENTAL

Molecular modeling and assembly of constructs

Numerical values of some molecular properties and modeling was accomplished utilizing ACD/ChemSketch modeling v. 10.00 (Advanced Chemistry Development, 110 Yonge Street, Toronto Ontario, M5C 1T4 Canada). Other properties: polar surface area, violations of Rule of 5, molecular volume, number of oxygens, nitrogens, amines, hydroxyls, etc were determined using Molinspiration (Molinspiration Chemiforatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic). In silico structure search for substituent replacement was accomplished using Chemical substructure and similarity search with MolCart Chemical Data Base (Molsoft L.L.C, 3366 North Torrey Pines Court, Suite 300, La Jolla, CA 92037 U S A).

Numerical analysis of multivariate data matrix

Statistical analysis of all numerical data was performed by Microsoft EXCEL (EXCEL 2003, copyright 1985-2003). Correlation analysis by Pearson r was done for some descriptors and was accomplished by GraphPad Software (GraphPad Instat v. 3.00 for Windows 95 GraphPad Software, San Diego California USA).

RESULTS AND DISCUSSION

Variation of structural substituents of biological active agents have been shown to have substantial beneficial affects on the pharmaceutical properties. Substituent modification can alter the druglikeness characteristics either favorably or destructively in terms of clinical efficacy. Considerable work has been already accomplished that correlate some structure modifications directly to important medicinal attributes such as bioavailability, lipid solubility, aqueous solubility, etc. Some investigators have shown a particular relevance of Log P, formula weight, and hydrogen bonding activity to the effective druglikeness of a compound. A screening or filtering of potential drug candidates is possible by statistical comparison to already proven but related medicinal compound molecular properties. This approach of filtering drug candidates improves the success rate for selection for drug trials evaluation and eventual full development.

One notable filtering method is known as the Rule of 5, where Log P, formula weight, and hydrogen bonding activity is taken to be some multiple of the numeric value of five. Specifically the criteria impute that violation of two or more of the parameters would signal problems in drug bioavailability. These criteria describe favorable druglikeness is found for attributes: 1) A Log P value of less than 5; 2) A formula weight less than 500grams/mole; 3) No more than 10 hydrogen bond acceptors (oxygen and nitrogen atoms); 4) No more
Doxorubicin

(A)

(B)

(C)

(D)

(E)

(F)

(G)
than 4 hydrogen bond donors (–OH and –NH$_2$). Doxorubicin is a complex molecule that intercalates the DNA molecule by way of the planar aromatic chromophore portion of the molecule and with the daunosamine sugar resting in a minor groove with accompanied interaction on the flanking base pairs$^{[1-3]}$. To pursue a useful modification of doxorubicin will require a planar chromophore region that effectively rests between two adjacent base pairs of DNA and a sugar substituent that successfully interacts with adjacent base pairs restraining the molecule in position.

The parent doxorubicin molecular structure as well as the derivatives formed from that scaffolding are shown in figure 1. All compounds retain the flat planar chromophore region of this intercalating agent for insertion between two bases of DNA. Drug B design features an imine group replacing the former primary amine group of the daunosamine sugar, a modification which brings on significant changes in important pharmaceutical properties. Drugs C, D, E, F, G, H, and I are a homologous series of derivatives having an alkoxy (–OR) replacing the former hydroxy ketone functional group of doxorubicin. Homologous series of compounds have a particular advantage of having predictable properties and extensive studies have shown that as the numeric series increase the medicinal activity also increases to a maximum of six or seven carbon chain length (–(CH$_2$)$_5$CH$_3$ or –(CH$_2$)$_6$CH$_3$)$^{[13]}$. Lengthening the carbon chain of the alkoxy group (–OR) produces substantial variation in the molecular properties that are considered influential in drug likeness (ie. Log P, formula weight, rotatable bonds, molecular volume, and water solubility).

Molecular properties (descriptors) for doxorubicin and derivatives were determined and presented in
TABLE 1. Several observations become immediately apparent for this assortment of anticancer agents, namely that the number of violations of the Rule of 5 remains at three, an expected outcome on account of variation occurs only on the daunosamine sugar (drug B) or the hydroxy ketone group. This is a known restriction for anthracyclines and explains part of the rational for intravenous administration of this agent. In this case the number of violations of Rule of 5 does not rule out the clear effectiveness as an antineoplastic agent. The number of oxygen and nitrogen atoms (hydrogen bond acceptors) remains constant at 12 throughout this assortment of compounds. The number of rotatable bonds increases as the length of the aliphatic alkyloxy substituent as does the formula weight, molecular volume, and Log P (becoming more lipophilic as the aliphatic alkyloxy group extends in length). The polar surface area of all the homologous series compounds (drugs C, D, E, F, G, H, I) remains constant at 195.084 Angstroms[2], which is a value that does not facilitate intestinal absorption[12]. Interestingly the water solubility of drug B is substantially higher at 390mg/Liter than that of doxorubicin itself at 92.84 mg/Liter.

This is apparently due to the substitution of a primary amine group with an imine group that is covalently bonded to the daunosamine sugar (Figure 1). As the length of the aliphatic alkyloxy substituent increases the water solubility continues to decrease, an expected outcome due to the increased lipophilic tendency of the aliphatic branch. Evaluation of correlation Pearson r for these descriptors reveal that Log P is directly correlated (r > 0.9500) to molecular weight, number of rotatable bonds, and molecular volume (coefficient of determination > 0.9025, showing account of more than 90% of variance). Descriptor Log P is inversely correlated to polar surface area, number of –OH and –NH (hydrogen bond donors), and water solubility.

Based solely on Log P numeric values, other significant observations can be determined for this group of compounds. Previous studies over the affluence of drug lipophilicity, measured as Log P, have shown that values of Log P that is 2 ± 0.7 may indicate potential penetrate the central nervous system[14], these including drug D, E, and F. A Log P value of 1.35 favors intestinal absorption, fulfilled by drug B. Colonic absorption is promoted by a Log P value of 1.32, fulfilled by drug B[14]. A Log P value between 3 to 4 promotes transdermal administration, which is fulfilled by drugs G and H.

Analogy among these derivatives to doxorubicin is expected and an example is presented in figure 2. The formula weight of the compounds increase and affect the relative relationship of Log P (1-octanol/water partitioning) and number or rotatable bonds. Seen in Figure 2 the Log P increases (drugs become more lipophilic) as the formula weight increases. Increase in lipophilicity suggests greater absorption into cell membrane by-layers. In addition, as the formula weight increase the number of rotatable bonds increase, suggesting some flexibility in molecular configuration.

Descriptor numerical values for these derivatives of doxorubicin confirms that drugs B, C, D, E, F, G, H, and I can be expected to express differentiation in pharmaceutical activity and medicinal action in the treatment of breast cancer.

### TABLE 1: Molecular Properties

<table>
<thead>
<tr>
<th>Drug</th>
<th>Log P</th>
<th>Polar surface area(A(^2))</th>
<th>Molecular Weight</th>
<th>Number of oxygens &amp; nitrogens</th>
<th>Number of -OH &amp; -NH</th>
<th>Violations of rule of five</th>
<th>Number of rotatable bonds</th>
<th>Molecular volume(A(^3))</th>
<th>Water Solubility(mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin,A</td>
<td>0.567</td>
<td>206.08</td>
<td>543.525</td>
<td>12</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>459.18</td>
<td>92.84</td>
</tr>
<tr>
<td>B</td>
<td>1.395</td>
<td>203.907</td>
<td>541.509</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>453.362</td>
<td>390</td>
</tr>
<tr>
<td>C</td>
<td>1.183</td>
<td>195.084</td>
<td>557.552</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>6</td>
<td>476.708</td>
<td>10.21</td>
</tr>
<tr>
<td>D</td>
<td>1.559</td>
<td>195.084</td>
<td>571.579</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>7</td>
<td>493.51</td>
<td>3.15</td>
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<tr>
<td>E</td>
<td>2.062</td>
<td>195.084</td>
<td>585.606</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>510.312</td>
<td>0.9714</td>
</tr>
<tr>
<td>F</td>
<td>2.621</td>
<td>195.084</td>
<td>599.633</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>9</td>
<td>527.114</td>
<td>0.2993</td>
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<tr>
<td>G</td>
<td>3.126</td>
<td>195.084</td>
<td>613.66</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>543.915</td>
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<tr>
<td>H</td>
<td>3.632</td>
<td>195.084</td>
<td>627.687</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>11</td>
<td>560.717</td>
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<tr>
<td>I</td>
<td>4.137</td>
<td>195.084</td>
<td>641.714</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>12</td>
<td>577.519</td>
<td>0.00873</td>
</tr>
</tbody>
</table>

A\(^2\) is Angstroms\(^2\), A\(^3\) is Angstroms\(^3\)
CONCLUSION

In summation, eight derivatives of doxorubicin were elucidated from the parent scaffolding of doxorubicin. Doxorubicin has been shown in previous studies to be effective in the treatment of metastatic breast cancer, solid tumors, and ovarian epithelial cancer. Disadvantages of doxorubicin include cardiotoxicity and poor intestinal absorption, necessitating intravenous administration. By forming derivatives of doxorubicin the Log P outcome suggests an improved potential for drug B (imine derivative) to accomplish intestinal absorption and colonic absorption. A Log P value between 3 to 4 promotes transdermal administration, which is fulfilled by drugs G and H. For the homologous series of drugs (C, D, E, F, G, H, and I) the increasing length of the aliphatic alkoxy substituent increases lipophilicity (increased Log P), increases molecular volume, increases formula weight, but decreases water solubility. Violations of the Rule of 5 remains at three for all compounds studied here. Be proposing modifications in the structure of doxorubicin it was possible to widely vary the Log P descriptor and potentially and beneficially alter the biological activity of these anthracycline antibiotics.

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REFERENCES