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Synthesis of novel anthracycline derivatives containing azido glycosides

Síntese de novos derivados de antraciclinas contendo azido glicosídeos

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ABSTRACT

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Anthracyclines are ranked among the most effective chemotherapeutics against cancer. They are glycoside drugs comprised by the aminosugar daunosamine linked to a hydroxyanthraquinone aglycone, and act by DNA-intercalation, oxidative stress generation and topoisomerase II poisoning. Regardless of their therapeutic value, multidrug resistance and severe cardiotoxicity are important limitations arising from anthracycline treatment, prompting the discovery of novel analogues, for instance through glycodiversification. This work aimed to exploit azido glycosides, to be combined with anthracycline aglycone and generate novel glycosides. In a semi-synthesis approach, both daunorubicinone and protected doxorubicinone were glycosylated with conveniently functionalised 2-azido glucosyl and galactosyl donors, as well as glycals. A screening of glycosylation protocols involved glycosyl chlorides, imidates and thioglycosides with the most successful promoters being $HgO/HgBr_2$ (4-52% yield) and TMSOTf (38-41%); for glucals and galactals, Au(I) and Cu(I) catalysts gave moderate yields (15-46%), but thiourea-phosphoric acid was the most efficient catalyst system (18-95%). Cleavage of protecting groups proved challenging, hampering and delaying the obtention of free glycosides. Upon deprotection, the glycosides obtained included glucoside **49** (13%), 2-azido glucoside **51** (34%), 2-deoxyglucoside **58** (11%), and 2-deoxygalactoside **61** (85%), all with the daunorubicin scaffold. In cell proliferation assays, glycosides **61α** and **61β** were tested against human cancer cell lines HeLa, MDA-MB-231 and MCF-7 and a model of healthy cells (HDF), with IC_{50} in the range of 27.1 to 74.6 μM for the α anomer, and higher than 250 μM for the β anomer. Preliminary studies with human cardiomyocytes derived from induced pluripotent stem cells were inconclusive to establish a cardiac toxicity experimental model.

Keywords: Anthracycline. Azido glycoside. Glycodiversification. Cytotoxicity. Cardiotoxicity

RESUMO

MARTINS TEIXEIRA, M. B. Síntese de novos derivados de antraciclinas contendo azido glicosídeos. 2018. 298 p. Tese (Doutorado). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2018.

Antraciclinas estão entre os mais eficazes quimioterápicos contra o cancer. São fármacos glycosídicos compostos pelo carboidrato daunosamina ligado a uma aglicona hidróxi antraquinona, e atuam por intercalação ao DNA, geração de estresse oxidative e envenenamento de topoisomerase II. Apesar de sua utilidade terapêutica, multirresistência e cardiotoxicidade grave são importantes limitações decorrentes do tratamento com antraciclinas, estimulando a descoberta de novos análogos, por exemplo através de glicodiversificação. Este trabalho objetivou explorar azido glicosídeos, a serem combinados com agliconas de antraciclinas para gerar novos glicosídeos. Em uma estratégia semi-sintética, daunorrubicinona e doxorrubicinona protegida foram glicosiladas com doadores 2-azido glucosídicos e -galactosídicos, além de glicais. Uma varredura de metodologias de glicosilação envolveu cloretos, imidatos e tioglicosídeos, sendo os promotores com melhores rendimentos $HgO/HgBr_2$ (4-52%) e TMSOTf (38-41%); para glucais e galactais, catalisadores de Au(I) and Cu(I) forneceram moderados rendimentos (15-46%), mas o sistema mais eficiente foi o organocatalisador de tiouréia e ácido fosfórico (18-95%). A clivagem dos grupos de proteção foi desafiadora, dificultando e atrasando a obtenção dos glicosídeos livres. Mediante desproteção, os glicosídeos obtidos incluíram glucosídeo **49** (13%), 2-azido glucosídeo **51** (34%), 2-desóxi glucosídeo **58** (11%) e 2-desóxi galactosídeo **61** (85%), todos com o esqueleto de daunorrubicina. Em ensaios de proliferação celular, os glicosídeos **61α** e **61β** foram testados em linhagens de células tumorais humanas HeLa, MDA-MB-231 e MCF-7 e um modelo de células sadias (HDF), com IC_{50} na faixa de 27.1 a 74.6 μM para o anômero α, e superior a 250 μM para o anômero β. Estudos preliminares com cardiomiócitos humanos derivados de células-tronco induzidas foram inconclusivos para estabelecer um modelo experimental de toxicidade cardíaca.

Palavras-chave: Antraciclina. Azido glicosídeo. Glicodiversificação. Citotoxicidade. Cardiotoxicidade

Introduction

1 INTRODUCTION

1.1 Cancer and anticancer treatment

The global cancer burden has been continually rising, with predictions to keep growing throughout the coming decades, associated with population ageing and contemporary lifestyle, especially in lower-resource countries that are undergoing major social, demographic, and economic transitions. According to the most current appraisals of cancer incidence and mortality worldwide, the World Health Organization (WHO) estimates more than 18 million new cases diagnosed in 2018, which is projected to increase by over 60% in the next two decades; and at least 9.6 million deaths from cancer during this year, placing the disease as the second leading cause of death globally.[1-3]

Cancer is characterised by abnormal cell transformation with unsuppressed growth and spreading, due to the gradual acquirement of cellular capabilities, such as limitless proliferation and evasion from control mechanisms, known as cancer hallmarks. Ultimately, such multistage process results from cumulative genetic mutations that dysregulate proto-oncogenes and tumour suppressor genes, causing genome instability. In turn, genomic alterations are multifactorial, ensued from the interaction between the individual genetic profile and external agents, including physical, chemical, and biological carcinogens.[4-6]

Among the nearly two hundred existing cancer types, the most common incidence sites are lung, breast, colorectum, prostate, stomach and liver. Although the classification of cancers is traditionally based on the organ of origin combined with histological typing, the increasing knowledge on tumour genomics is providing a deeper refinement of cancer complexity at the molecular level, which is expected to provide better prognostic power and more precisely targeted therapies.[3, 7]

A milestone in anticancer therapy was the discovery of cytotoxic agents in the last century, improving survival rates and the quality of life for cancer patients.[6, 8] Currently, there is a vast therapeutic armoury available for the treatment of a variety of cancer types: alkylating agents, intercalators, antimetabolites, antimitotic, enzyme inhibitors, hormone antagonists, in addition to immuno and gene therapies (Figure 1).

Underlying many of these antineoplastic classes is the disruption of nuclear mechanisms, which kills cancer cells by strategically exploiting their rapid replication as a selectivity feature. However, these drugs also affect healthy tissues that inherently have a

high cell renovation rate, causing recurrent side effects, such as bone marrow suppression, hair loss, mucosal irritation, gastrointestinal disturbances, and many others.[9]

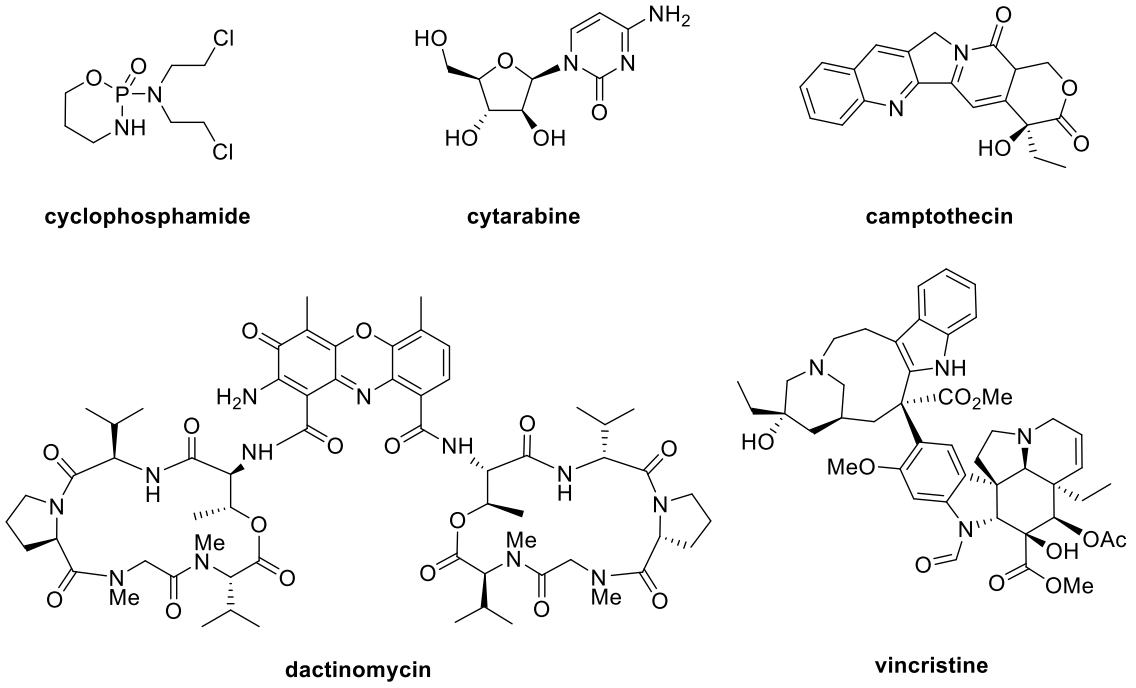

Figure 1. Selected examples of cytotoxic drugs affecting cell replication processes. Cyclofosfamide, a DNA-alkylating agent; cytarabine, a DNA polymerase inhibitor and chain terminator; camptothecin: a topoisomerase I poison; dactinomycin, a DNA intercalator; vincristine, a tubulin-polymerisation inhibitor;

1.2 Anthracyclines

Anthracyclines comprise a class of natural antibiotics among the most effective antineoplastic agents in current clinical use, with few unresponsive cancer types. The first representative compounds of this family were isolated from a mutant strain of the actinobacteria *Streptomyces peucetius* by Arcamone and Di Marco, as part of a systematic search for antitumour agents by an industrial company (Farmitalia Research Laboratories of Milan). Daunorubicin (**DAU**) was described in 1964, followed by doxorubicin (**DOX**) in 1969, when they used to be named "daunomycin" and "adriamycin", respectively (Figure 2). They were soon tested clinically, achieved registration in the early 1970s, and have been marketed since then, becoming the prototypes of the anthracycline class.[10-13]

After half a century of their discovery, the so-called first-generation anthracyclines are still frequently prescribed today and persist as a mainstay in many chemotherapeutic schemes. Ranked among the most potent and widely useful anticancer drugs, **DAU** and **DOX** are

consistently listed by WHO as essential medicines for cancer treatment. While daunorubicin is indicated against acute lymphoblastic and myeloblastic leukaemias, doxorubicin is more active on lymphomas, sarcomas, and a broad spectrum of solid tumours, including breast, lung, bladder, bone, and cervical cancers. [6, 13-15]

Such a distinct range of anticancer activity between daunorubicin and doxorubicin is defined by a minor structural difference, as shown in Figure 2. Overproduced by a number of strains, **DAU** is the immediate biosynthetic precursor of **DOX** in *Streptomyces peucetius caesius* ATCC 27952, the single strain reported to produce doxorubicin, and which complete genome was recently sequenced. However, to date, it proved not to be amenable for scaling up, as strain improvement techniques and genetic engineering have not delivered a cost-effective fermentation process for **DOX** yet. Therefore, on the industrial scale, doxorubicin is prepared by semisynthesis from daunorubicin, through a chemical bromination at C-14, followed by displacement of the bromine by hydroxide with mild base treatment.[13, 16, 17]

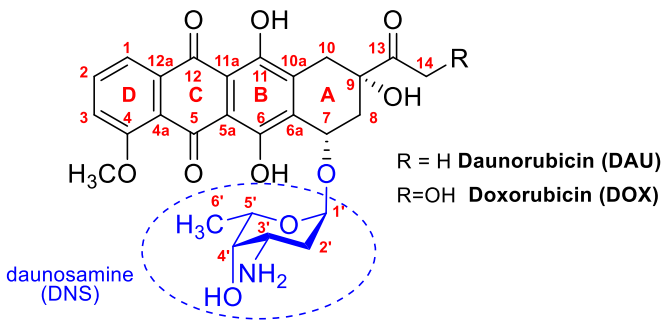

Figure 2. The first generation of anthracyclines: daunorubicin and doxorubicin. The 3-amino-2-deoxyglycosyl unit "daunosamine" is stressed. Conventional structural numbering and naming in accordance with Brockmann (1963), who described the isolation and elucidation of the first anthracycline compounds.[18]

Both daunorubicin and doxorubicin are natural product glycosides derived from microbial polyketide biogenetic intermediates, sharing a polyhydroxy anthraquinone skeleton (rings B, C and D) fused to a fourth saturate substituted ring (A), altogether corresponding to the aglycone moiety. The side chain at C-9 differentiates **DOX** from **DAU**, the former holding an extra hydroxyl group at position 14. The hydroxylated tricyclic quinone system is the chromophore responsible for the red to orange colour characteristic of anthracyclines, which absorb around the 480 nm range of the visible light spectrum. Attached to ring A at the benzylic position 7 is the unique glycone moiety 3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranose, also known simply as daunosamine (**DNS**), an unusual amino deoxy sugar conserved in tens of anthracycline congeners and considered crucial for their anticancer activity.[13, 18]

Many other bioactive natural products contain glycosides, in which the carbohydrate residues are often essential, as illustrated in Figure 3. Some types of carbohydrates are repeatedly present in various classes of glycoconjugates, such as 2-deoxysugars,[19] found in antiparasitic macrocyclic lactones (ivermectin B_{1a}), aureolic acid antibiotics (chromomycin A3), and cardiotonic agents (digoxin), for example. In addition, aminosugars, besides occurring most abundantly in structural polysaccharides, such as glucosamine in chitin and galactosamine in chondroitin, also exist in many rare and complex natural product glycosides, as is the case of 3-amino sugars in antibiotics like vancomycin, amphotericin B and erythromycin (Figure 3). Cytotoxic anthracyclines combine both structural features enclosed in the unique 3-amino-2-deoxyglycosyl residue daunosamine.[20]

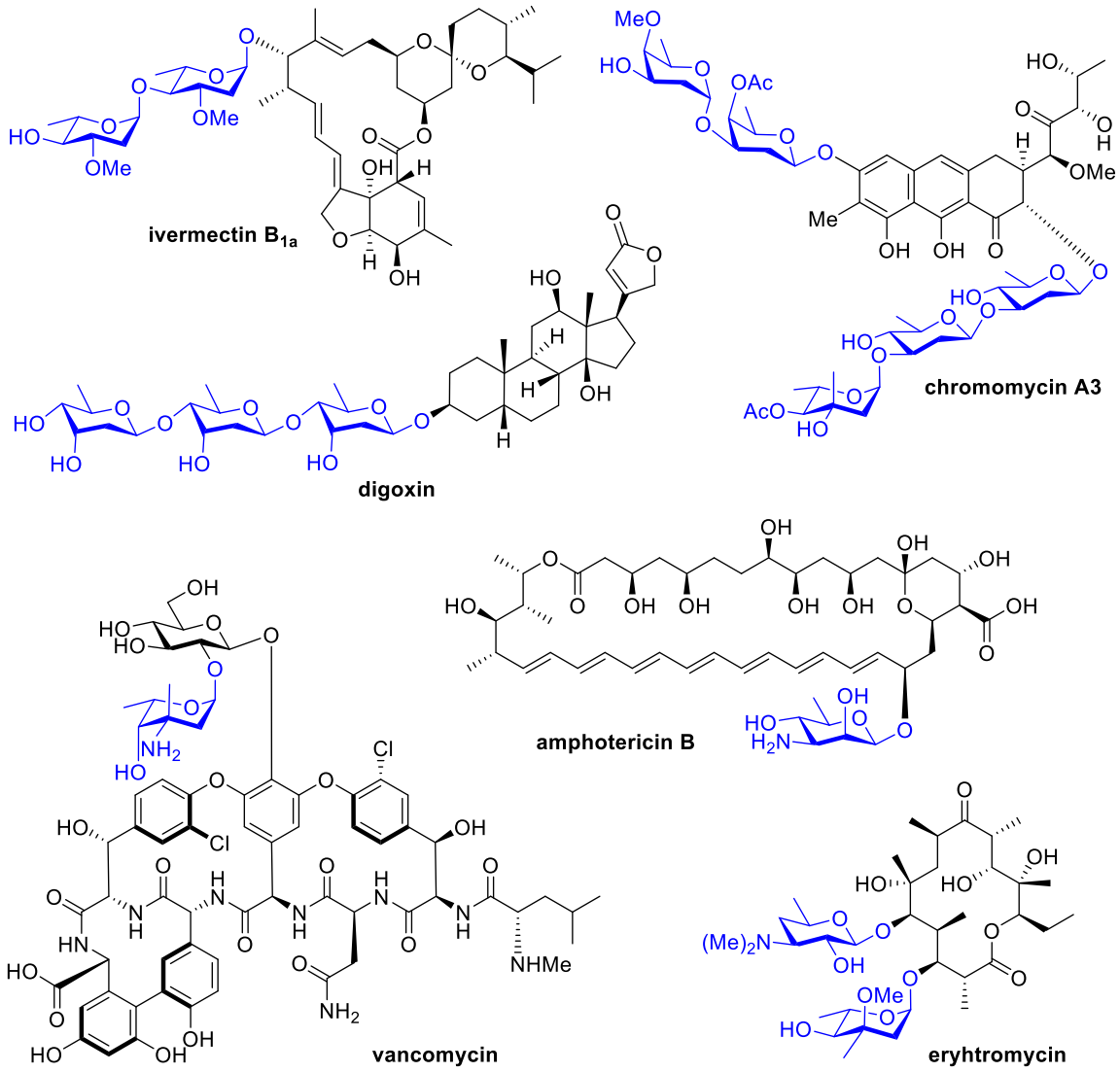

Figure 3. Selected examples of glycoside drugs containing 2-deoxy and 3-amino-3-deoxy carbohydrates, highlighting the glycones.

1.2.1 Mechanisms of action and structure-activity relationship considerations

Anthracyclines cytotoxic activity results from a strong inhibitory effect on nucleic acid synthesis, but there has been substantial controversy and debate over their exact molecular mechanisms of action. It is now generally recognised that anthracyclines act through a combination of multiple mechanisms and that the most consistent are, namely: induction of oxidative stress, intercalation into DNA, and more importantly, poisoning of topoisomerase II enzyme (Top2).[15, 21]

The formation of reactive oxygen species (ROS) induced by anthracyclines can be initiated by one-electron reductions of either the quinone into semi-quinone or chelated ferrous into ferric ions. These electron transfers trigger enzyme-mediated reduction-oxidation (redox) cycles that ultimately produce very destructive hydroxyl radicals, causing protein alkylation, lipid peroxidation, and particularly direct DNA damage and cross-links, followed by all downstream cellular cascades leading to cell death (Figure 4). Interestingly, the ability of anthracyclines to boost ROS production is also largely associated with their toxic effects, predominantly on myocardial tissue.[15, 22, 23]

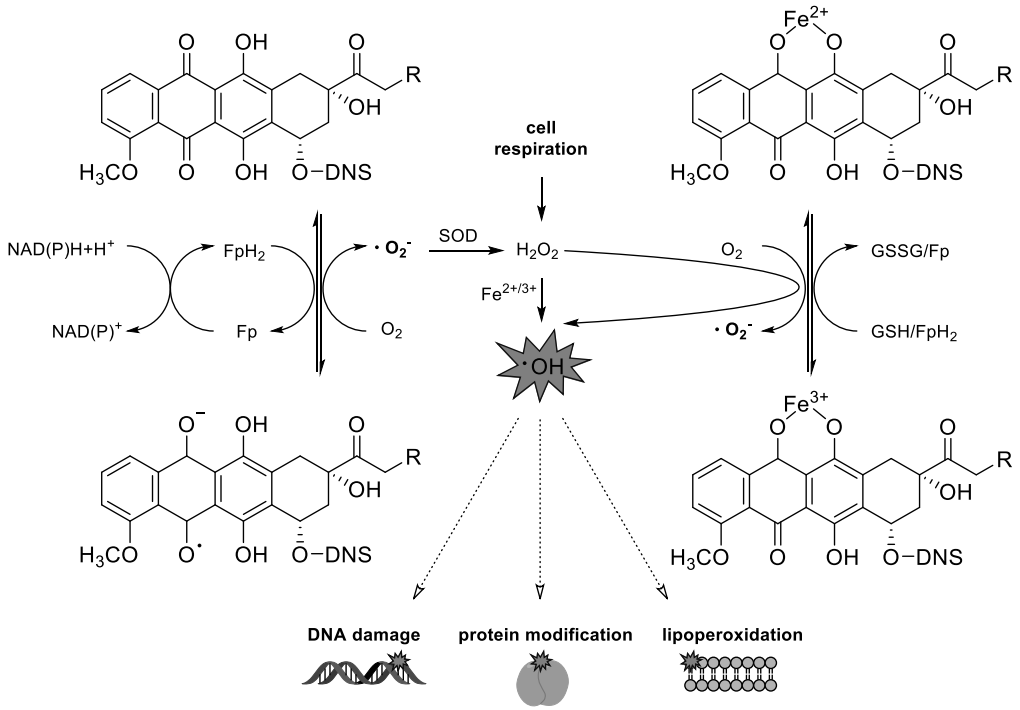

Figure 4. Schematic main pathways of anthracycline-induced and iron catalysed oxidative stress generation. Fp: flavoprotein, GSH/GSSH: reduced/oxidised glutathione, NAD(P): nicotinamide adenine dinucleotide (phosphate), SOD: superoxide dismutase. Adapted from Sterba (2013).[22]

Owing to their aglycone planar structure, comprised by a polyaromatic system, anthracyclines are capable of intercalating DNA. More specifically, rings B and C from the anthraquinone moiety stack between neighbouring base pairs, pushing them apart to result in bidirectional positive torsion of the double helix. Ring D occupies the major groove, while ring A and daunosamine project into the minor groove, as shown by the interaction of doxorubicin with a short DNA sequence in a crystalline structure, illustrated in Figure 5.[24, 25] Although intercalation itself can distort DNA and possibly interfere with the nuclear functions, it is considered necessary but not sufficient for the optimal cytotoxic activity of the anthracyclines, in particular at the *in vivo* concentrations elicited by therapeutic doses.[15, 22]

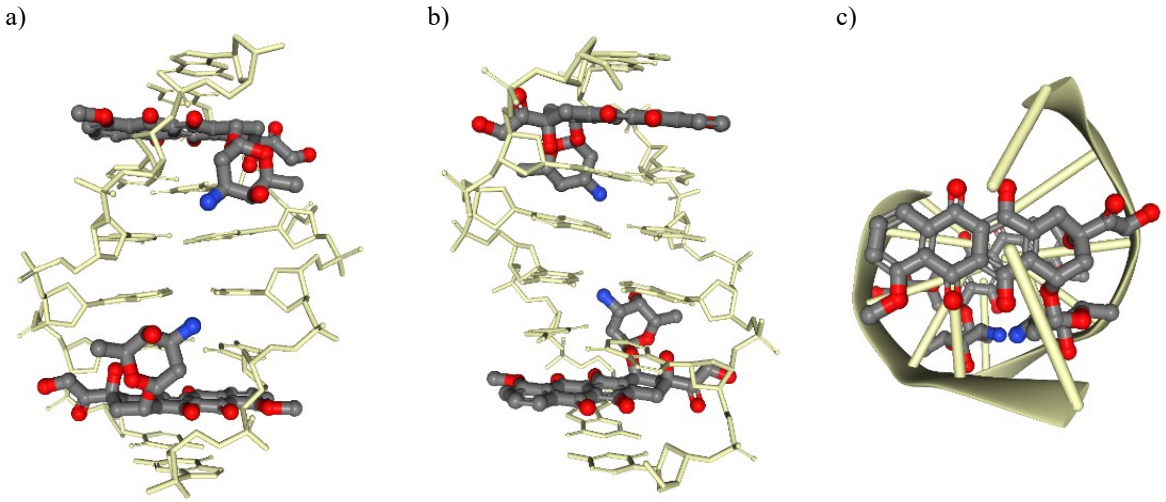

Figure 5. Crystallographic complex of two doxorubicin molecules with DNA sequence CGATCG, PDB 1P20. Adapted from [25] Lateral views from the minor groove (a) and major groove (b) perspectives; c) helix axial view projected through the anthracycline plane. Illustrations generated with web-based NGL Viewer.[26]

Intercalation becomes essential as part of the primary mechanism of action of anthracyclines, the poisoning of topoisomerase II enzyme. Observing other Top2 poisons, an obvious shared structural feature is the polyaromatic intercalating moieties (Figure 6).[27] Topoisomerase II is a nuclear enzyme that adjusts the torsion state of DNA, which tends to form supercoiled structures during replication and transcription over the cell cycle. To relief supercoiling tension, Top2 promotes transient breaks in both strands of a duplex DNA, passing an intact DNA double helix through the open gap, and then reseals the broken strands. In specific recognition sites, Top2 cleaves one phosphodiester bond on each strand of DNA and covalently binds to the 5′ OH of DNA backbone through the active site tyrosyl group, stabilising the broken strands in a cleavable complex. After unknotting, the enzyme promotes relegation of the broken strands and releases the restored duplex DNA (Figure 7).[27-29]

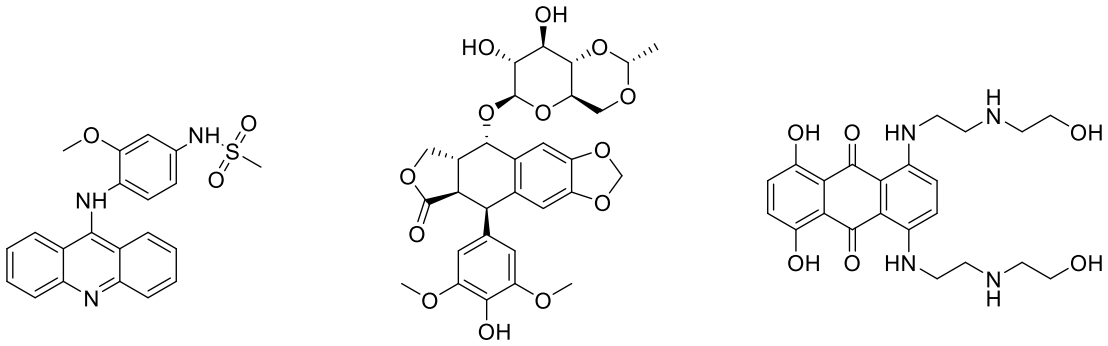

Figure 6. Anticancer drugs that target topoisomerase II. Intercalating moieties are essential for the poisoning of Top2 and are conserved in different poisons.

Anthracyclines stabilise the cleavable complex between Top2 and DNA, trapping the covalent intermediate into a ternary drug-DNA-enzyme complex, which prevents topoisomerase from properly regenerating the broken phosphodiester bonds. These drugs subvert the physiological enzyme functions, converting topoisomerase into a DNA-breaking nuclease, which leads to genomic instability and triggers apoptotic cell death.[15, 23, 30-32]

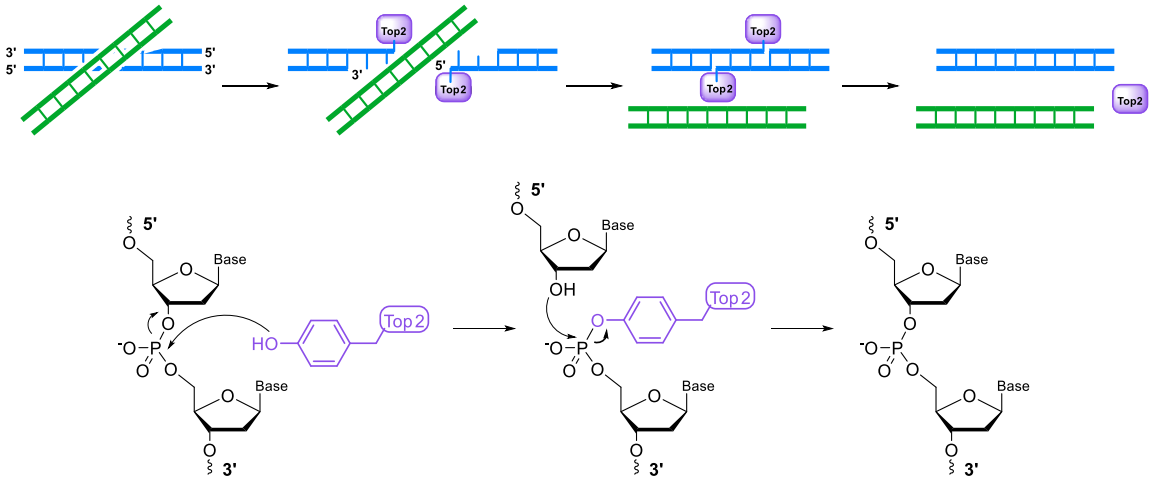

Figure 7. Catalytic mechanism of topoisomerase II. A transesterification occurs between the enzyme tyrosyl residue and DNA phosphodiester, breaking the DNA backbone bond and the forming a covalent enzyme–DNA intermediate. It allows another DNA chain to cross, after which the reversal reaction re-establishes the phosphodiester bond. Anthracyclines stabilise the intermediate in a ternary complex, poisoning the enzyme.

In this mechanism of action, while the planar aromatic aglycone stacks between DNA base pairs, the glycosyl unit plays a crucial role, projecting through the helix groove to bridge the interaction with topoisomerase II and stabilise the ternary complex, the basic amino group being determinant for the stabilisation and binding affinity (Figure 8). Changing the

configuration of daunosamine to the β anomer modifies the mode of binding. Blocking the amino function with amide group reduces cytotoxicity, but the replacement at C-3′ with a small group such as hydroxyl retains comparable activity while averting multidrug resistance.[31, 33] Conformational and crystallographic studies show that daunosamine is the most flexible domain in anthracyclines, possibly adopting numerous stable conformations for an optimal fit of the sugar at the interface between DNA and topoisomerase. Thus, modifications in the amino sugar are not only tolerable regarding bioactivity, but also of potential interest in drug development.[33, 34]

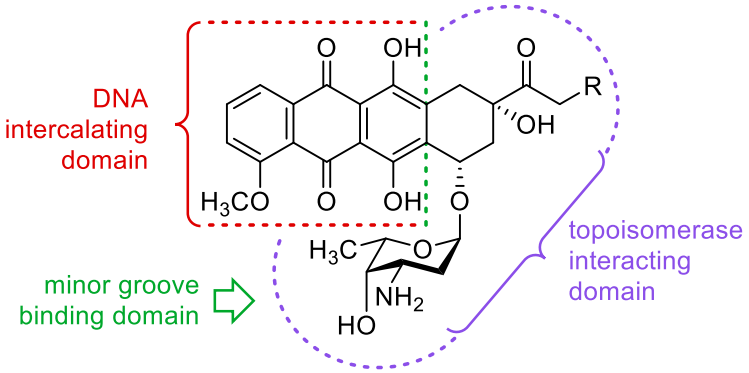

Figure 8. Anthracycline domains relevant for the binding to DNA and topoisomerase enzyme, and consequently to the pharmacological activity.

1.2.2 Limitations of anthracyclines

Despite their widely acknowledged efficacy, anthracyclines clinical use is significantly limited by their toxicity, the most serious side effects manifesting in the myocardial tissue. Chronic cardiomyopathy is dose-dependent and develops in 5-20% of patients receiving cumulative doses higher than 500 mg/m^2 of doxorubicin and 900 mg/m^2 of daunorubicin. Within months or years after treatment, symptomatic congestive heart failure evolves, which is severe, progressive, irreversible, usually refractory to conventional therapy, and associated with high mortality rates.[6, 15, 35]

The mechanisms underlying anthracycline-induced cardiotoxicity are complex, multifactorial, and not fully elucidated yet. The most accepted is the generation of reactive oxygen species in cardiomyocytes, which cells are particularly susceptible to free radical damage due to their deficiency in catalase and dismutase enzymes. ROS are overproduced by one-electron reductions, mediated by chelated iron and subsequent redox cycles of

flavoproteins (NAD(P)H oxidoreductases) (Figure 4). The biotransformation of anthracyclines involves the two-electron reduction of the C-13 carbonyl into secondary alcohol (doxorubicinol or daunorubicinol), via sequential metabolism by aldo-ketoreductase and carbonyl reductase enzymes. These metabolites greatly accumulate in cardiomyocytes, and are particularly harmful to these cells, especially regarding iron-dependent mechanisms. Mitochondrial dysfunction, calcium misbalance and apoptosis induction are other alterations involved in anthracycline cardiotoxicity.[15, 35-39] Moreover, Top2β isoform present in cardiomyocytes was shown to mediate doxorubicin-induced heart damage, whilst Top2α isoform overexpressed in cancers cells is the molecular target of anthracycline therapeutic activity.[40, 41]

A second major limitation related to anthracyclines is the development of resistance by certain cancer cells, which can be rendered by several specific inherent or acquired factors. For example reduced expression or activity of topoisomerase II, decreasing enzyme-mediated DNA damage; overexpression of superoxide dismutase enzyme, reinforcing cell defences against oxidative stress; suppression or mutation of p53, affecting apoptotic signalling pathways to prevent cell death. More importantly, multidrug resistance through altered membrane transport is the primary mechanism behind resistance to anthracyclines, exploited by cancer cells to evade the toxic effects of chemotherapeutics.[42]

In multidrug resistant cells, active drug efflux is mediated by proteins of the ATP binding cassette family, markedly P-glycoprotein (P-gp) and MRP transporters, which are overexpressed on the cell surface. These efflux pumps are capable of recognising and expelling not only the drug which induced the resistance but a great diversity of drugs without any structural or functional similarity. Anthracyclines are known substrates for such nonspecific transporters, decreasing intracellular drug concentrations, diminishing its effectiveness and thus resulting in drug resistance. [43-45]

1.2.3 New generation anthracycline and other analogues

In the pursuit for better anthracyclines that could overcome the cardiotoxicity and resistance limitations of the parent drugs, thousands of analogues have been isolated or synthesised, over the past decades, including modifications in the tetracyclic rings, the side chain and the amino sugar. The initial rationale was to avoid alterations in the general architecture of the molecule and the chemical functionalities, to retain the structural requirements for action, giving rise to second-generation anthracyclines, among which epirubicin and idarubicin proved to be clinically useful and are the most prominent derivatives.[46]

Epirubicin is a doxorubicin analogue in which the configuration of the C-4′ hydroxyl group is inverted, so the substituent lies in the equatorial orientation (Figure 9). It was first synthesised by glycosylation of the aglycone with a protected L-acosamine (3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranose), but further development allowed to perform epimerisation directly on the anthracycline glycoside. Epirubicin is about 30% less cardiotoxic than **DOX**, owing to differences in the pharmacokinetic profile, through extensive detoxification as a 4′ glucuronide, without affecting the antitumour properties of the drug. Because it is better tolerated, cumulative doses threshold is roughly twice as much as for doxorubicin.[15, 46]

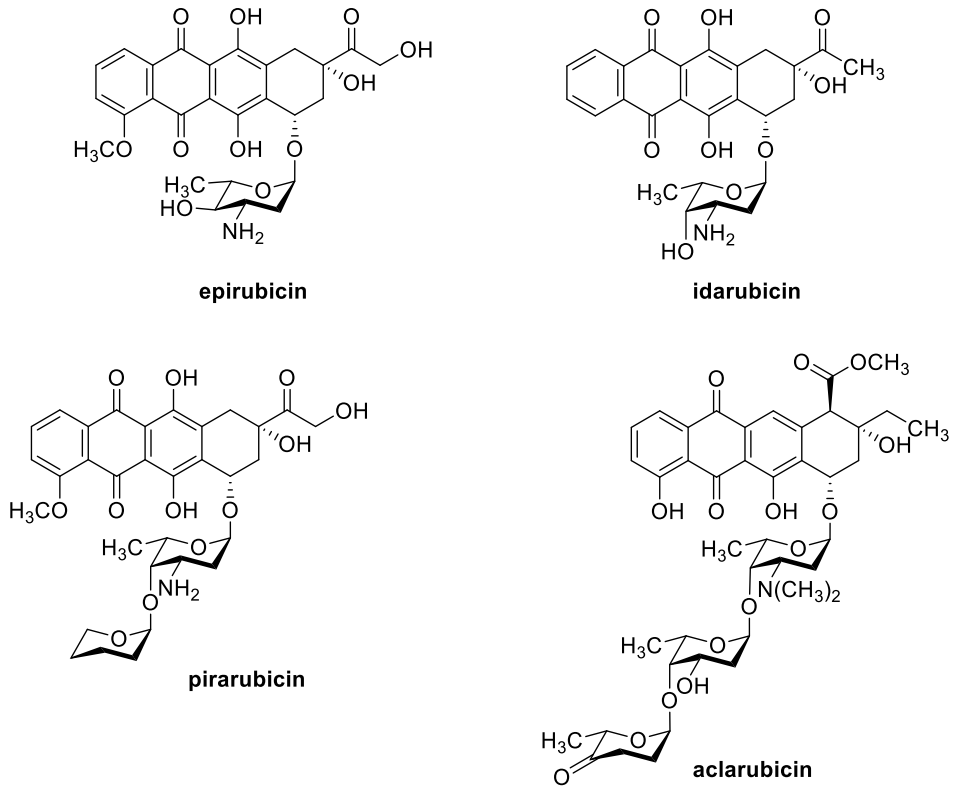

Figure 9. Second-generation anthracyclines.

Idarubicin is the 4-demethoxydaunorubicin (Figure 9), prepared through glycosylation of the non-natural 4-demethoxydaunorubicinone. Originally produced by total synthesis, the aglycone could also be obtained semi-synthetically from daunorubicinone. This drug can be administered orally and shows a broader spectrum of activity compared to **DAU**, including not only leukaemias but also some solid tumours, probably by increased lipophilicity and cellular uptake.[15, 46]

Despite the numerous efforts, only some other analogues reached the stage of clinical development and even fewer achieved approval for marketing.[15, 35] Semi-synthetic pirarubicin (4′-tetrahydropyranyl-doxorubicin, Figure 9) is considered less cardiotoxic than **DOX** and exhibits activity against some doxorubicin-resistant cell lines. Aclarubicin is claimed to have reduced cardiotoxicity than **DOX** and **DAU**, but only moderate improvement in terms of drug resistance. This trisaccharide anthracycline from natural origin (*Streptomyces galilaeus*), comprised by a variant aglycone structure (Figure 9), has different mechanisms of action, including inhibition of Top2 before DNA breakage and histone eviction. These drugs became registered only in a few countries, and do not play a significant role in global terms.[8, 15, 35, 47, 48]

As for the third-generation anthracyclines undergoing clinical development, some are emerging as promising drug candidates. Nemorubicin (PNU 152243) is a semi-synthetic doxorubicin derivative having a more lipophilic 2-(*S*)-methoxy-4-morpholinyl at the 3′ position (Figure 10), discovered by lead optimisation within a series of morpholinyl anthracyclines.[49] In the preclinical phase, it was intensely potent, active against drug-resistant cell lines and xenografts, and not cardiotoxic. It is believed to be bioactivated by P450 CYP3A and to induce DNA strand breaks primarily through topoisomerase I cleavage.[50-52]

Sabarubicin (MEN 10755) derives from the glycosylation of 4-demethoxy-doxorubicinone with a disaccharide, resulting in a glycoside in which a 2,6-dideoxy-α-L-*lyxo*-hexopyranosyl residue is positioned between the aglycone and daunosamine (Figure 10), differently from the natural anthracycline disaccharides, which carry the aminosugar as the first moiety directly attached to the aglycone. The elongated glycone permits the second sugar to interact both at the minor groove and with a DNA base. It is less cardiotoxic than doxorubicin, but it does not show the ability to overcome transport-mediated resistance. [46, 47, 52]

Amrubicin (SM-5887) is a totally synthetic anthracycline analogue, developed through a simplification approach. In comparison with daunorubicin, it lacks the 4-methoxy group of the aglycone and the 3′ amino group of the carbohydrate, which is a minimalist version of daunosamine. On the other hand, an amino group appears at position 9, in replacement to the hydroxyl group. It is a prodrug, with the amrubicinol (13-OH) metabolite being more cytotoxic than the parent drug. Overall, it is less cardiotoxic than doxorubicin and epirubicin.[52, 53]

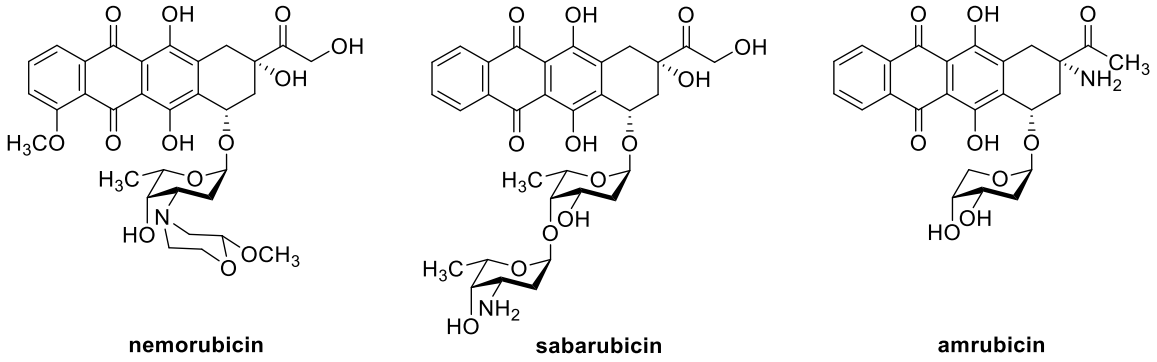

Figure 10. Third-generation anthracyclines.

Specific changes, substituting the amino function in daunosamine by azide, for instance, demonstrated to overcome resistance in specific cancer cells. The 3′-azido derivatives of daunorubicin, doxorubicin and epirubicin (Figure 11) were synthesised from the parent drug and retained antiproliferative activity against drug-sensitive human cancer cell lines of leukaemia (K562) and breast cancer (MCF-7). The first two compounds were further active against drug-resistant cell lines K562/Dox and MCF-7/Dnr, by averting P-glycoprotein binding and active efflux, with consequential intracellular accumulation. Flow cytometry and molecular modelling showed that the chemical modification, from positively ionisable amino group to the more electron-dense and linearly arranged azido group, abolished key interactions with P-glycoprotein. These azides were no longer substrates to P-gp efflux transporter, and were considered new leads for the development of innovative anthracyclines[43, 44, 54, 55] Although the azido group is not found in natural products, it is present in many bioactive compounds, including approved drugs such as azidocillin and azidamfenicol antibacterials, and zidovudine antiviral.

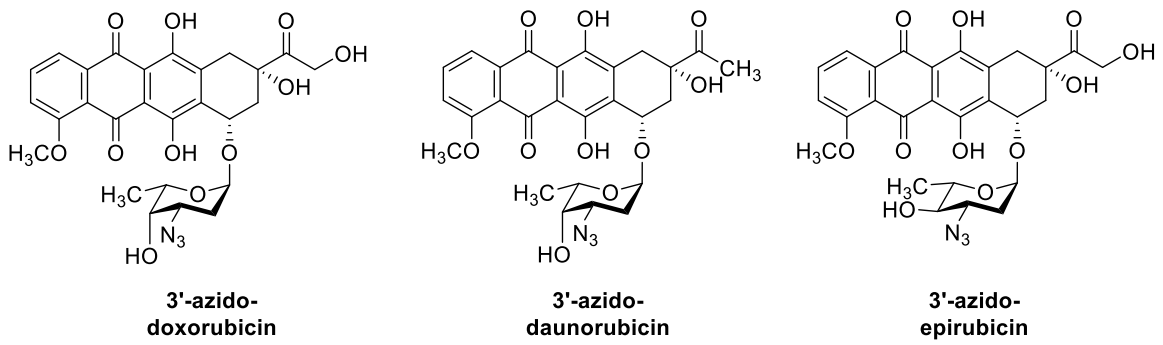

Figure 11. Anthracycline 3′-azido derivatives, some of which can overcome P-gp mediated resistance.

1.3 Glycodiversification and glycosylation reactions

The discovery of novel anthracyclines with wider anticancer spectrum, better heart tolerability and less prone to resistance remains a necessity. Many of the structural variations in new generation anthracyclines took place on the carbohydrate moiety, indicating that the glycone could be successfully replaced without losing the anticancer activity, and at the same time modulating other properties, such as pharmacokinetics, toxicity, and resistance profile. Because anthracyclines are glycoside drugs with demonstrated tolerance to glycone modification, they are promising candidates to the so-called glycodiversification approach. This stands for the replacement of the carbohydrate unit of a bioactive glycoside by sequential glycosidic cleavage, protection, re-glycosylation and deprotection steps, enabling the access to series of glycosides of interest (Figure 12).[56, 57]

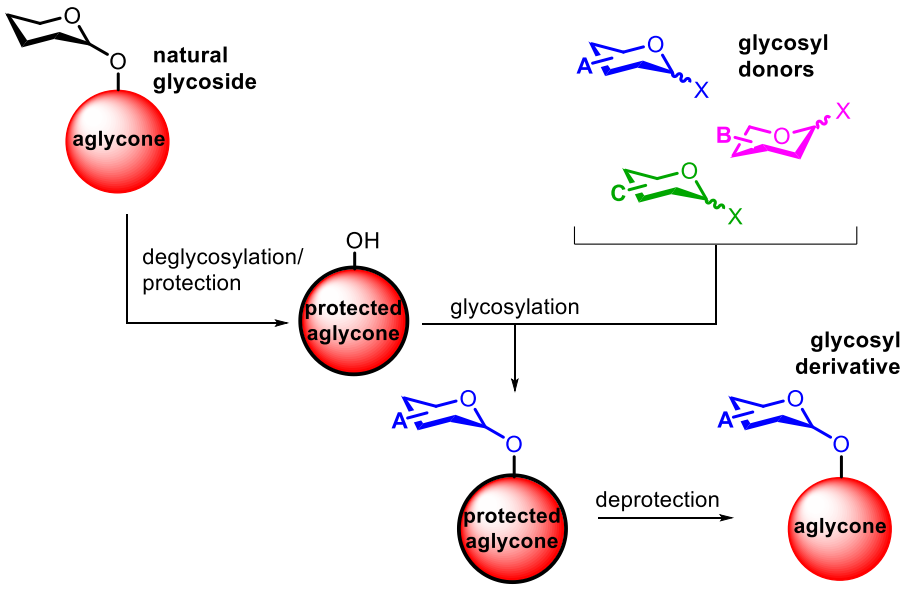

Figure 12. Glycodiversification strategy of natural glycosides, enabling the synthesis of libraries of novel glycoside derivatives. Adapted from Ritter (2003).[57]

This strategy is benefited by the possibility to work out the complexity of the carbohydrate separately, avoiding the valuable polyfunctional aglycone being exposed to unfavourable reaction conditions. Glycodiversification has been successfully applied to several classes of glycoconjugate drugs, such as the macrolide antibiotic erythromycin, the glycopeptide antibiotic vancomycin, the antiparasitic avermectins and the antifungal polyene amphotericin B (Figure 3).[20, 56, 57]

Based on this fruitful approach, it would be interesting to explore a variety of sugars in combination with anthracycline aglycones, with the glycosylation reaction playing a central role in connecting the two moieties. The chemical formation of a glycosidic bond is generally recognised as a challenging reaction, involving the linkage of a glycosyl acceptor, usually through a nucleophilic hydroxyl group, to the electrophilic anomeric carbon of a glycosyl donor equipped with a leaving group. A multitude of factors affects the efficiency and stereoselectivity of the glycosylation reaction, including the configuration of the carbohydrate moiety, the protecting groups on the substituents, the anomeric leaving group, the promoter system, the solvents, etc.[58]

The most important outcome to control in a glycosylation reaction is the stereoselectivity of the glycosyl bond. It is primarily governed by the anomeric effect, which is a stereoelectronic factor describing the tendency of a polar C-1 substituent, adjacent to the oxygen atom in the tetrahydropyran ring, to prefer the axial orientation instead of the equatorial one, despite the latter being favoured by lesser steric hindrance. These improbable observations can be rationalised by the following orbital interaction and electrostatic models. The hyperconjugation of one of the non-bonding electron pairs from the endocyclic oxygen atom with the anti-ligand orbital σ* from the anomeric carbon provides electronic stabilisation. The superimposition is efficient when the orbitals are parallel, which is only enabled when the anomeric bond is in the axial orientation, and the lowest unoccupied molecular orbital has a syn-periplanar relationship with one of the oxygen n orbitals (Figure 13a). Electrostatically, the dipole-dipole interaction between the endocyclic oxygen lone pairs and the electrons of the anomeric substituent is strongly repulsive when it is positioned in the equatorial orientation, but these destabilising interactions do not exist when it is in the axial orientation, and the dipoles are opposite (Figure 13b). [59, 60]

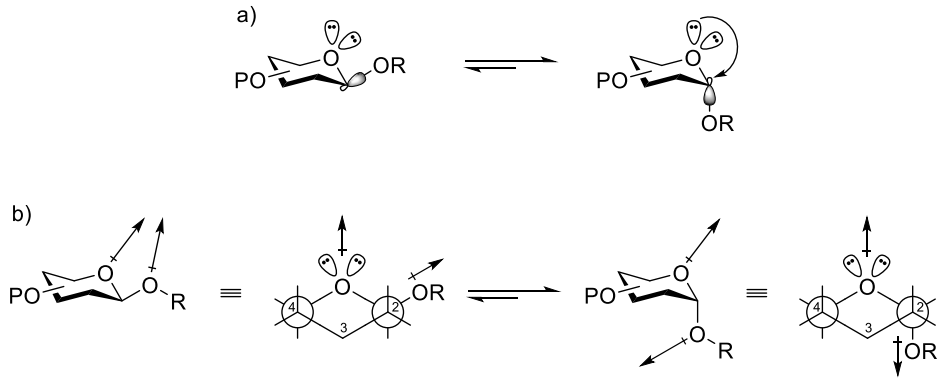

Figure 13. The anomeric effect explained by a) the orbital interaction model, and b) the electrostatic model.

In some cases, the anomeric effect can be outrivaled by other factors, reversing the stereo preference of the forming glycosidic bond from the axial to the equatorial orientation. The most relevant of these factors is known as the neighbouring group participation, in which the substituent at C-2 influences the outcome of the glycosylation reaction. After anomeric activation upon the departure of the leaving group, the anomeric effect prevails if the neighbouring group is non-participant, as is the case of ethers, silyl ethers and azides, and the resulting glycoside is predominantly axial (Figure 14a). When the neighbouring group holds an acyl functionality, such as esters, amides and carbamates, that can stabilise the transient cation through a dioxolenium- or oxazoline-like intermediate, the bottom face is occupied, and the nucleophile can only attack by the top face. This effect is also called anchimeric assistance and results mostly in equatorial-oriented glycoside with high stereoselectivity (Figure 14b). In spite of these directing effects, it is noteworthy that they are not absolute, and commonly a small amount of the opposite anomer is also produced, which can be negligible or not. [60, 61]

Protecting groups in the glycosyl donor, which have the primary function of preventing undesired side reactions, can also interfere by rendering the glycosyl donor more reactive ("armed", with electron donating groups) or less reactive ("disarmed", with electron withdrawing groups), or even influence on the conformation of the intermediate through steric effects, favouring a particular stereo outcome.[60, 62] As every glycosylation reaction is unique, a balance of these factors, combined with solvent, temperature, selection of anomeric leaving group and activation reagents, must be individually optimised.[60, 63, 64]

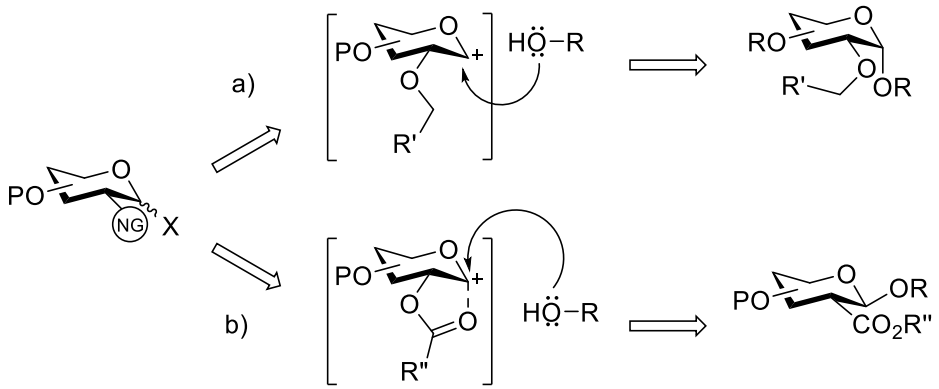

Figure 14. Neighbouring group effect on the glycosylation reaction stereoselectivity. a) Non-participating group at C-2 does not influence, and the anomeric effect governs the formation of the axial glycoside. b) Participating group at C-2 provides anchimeric assistance, reversing the stereoselectivity to the formation of the equatorial glycoside. NG: neighbouring group; P: protecting group; R, R′, R″: alkyl/aryl.

Encouraged by the therapeutic potential of anthracyclines as anticancer agents, and the possibility to develop novel derivatives that could retain antitumor efficacy with reduced toxicity and resistance, given their comprehensive structure-activity relationship, this work envisioned to exploit glycosylation reactions within a glycodiversification strategy to synthesise novel anthracycline derivatives coupled to azido glycosides.

Conclusion

5 CONCLUSION

The proposed glycodiversification route, involving cleavage of the glycosidic bond in daunorubicin and doxorubicin, the regioselective protection of latter, and the reaction with glycosyl moieties allowed to get a series of novel anthracycline protected glycosides, including 2-azido glycosides **45**, **46**, **47**, **50**, **52**, 2-deoxyglycosides **53**, **54**, **55**, **57**, **59**, **60**, and 6-azido-2,6-dideoxy glycoside **56**, achieving and extending the structural diversity originally planned.

The best glycosylation methodologies were the trimethylsilyl trifluoromethane-sulphonate catalytic activation of 2-azido glycosyl imidates, and the cooperative thiourea-phosphoric acid organocatalysis for glycal donors.

Deprotection of compounds **50**, **57** and **60** afforded, respectively, 2-azido glucoside **51**, the daunorubicin counterpart of target compound **1**, 2-deoxyglucoside **58** and 2-deoxygalactoside **61**. Some deprotection reactions are to be optimised, in order to produce a variety of final products from the available protected glycosides, including the deprotected form of 6-azido galactoside **56**, the structurally closest analogue of target compound **2**. The synthesis of 3′-azido-anthracyclines **3** and **4** was successfully reproduced from literature protocols, to serve as a comparison to the novel target compounds.

3′-azido-doxorubicin **3** showed comparable potency to the parent doxorubicin in antiproliferative assays against A431 cells. Compound **61α** was 10 to 1000 fold less potent than the parent daunorubicin in antiproliferative assays against HeLa, MDA-MB-231 e MCF-7 cell lines, reinforcing the interest for testing the azido-containing derivatives against the same panel of cancer cells.

A model of human cardiomyocytes derived from induced pluripotent stem cells could not be standardised for evaluating the synthesised compounds. An adequate experimental model still lacks for the assessment of their cardiotoxicity, which is to be addressed by means of collaboration.

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