

## Recent developments in the field of antitumour anthracyclines

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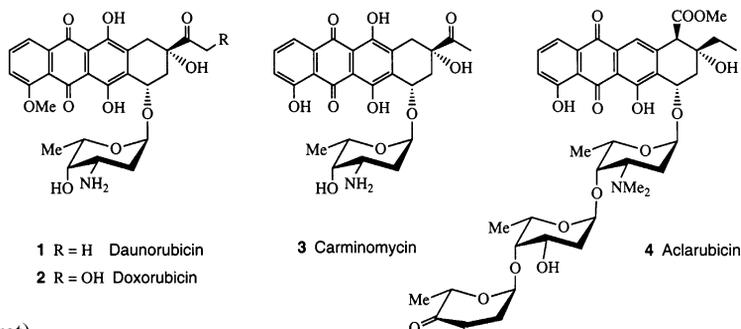
anthracyclines / antitumour / cardiotoxicity / targeting / A.D.E.P.T.

The anthracycline antibiotics daunorubicin (**1**) and doxorubicin (**2**) have been introduced in clinical use for more than 30 years for the treatment of a wide variety of cancers such as acute myeloid leukaemia, and, in the case of doxorubicin, a diversity of solid tumours [1]. Used as single agents or in combination therapy, they are the components of adjuvant, curative, as well as palliative treatments.

Despite their extensive clinical utilisation, their mechanism of action has been the subject of controversy. A critical evaluation of these has recently been proposed [2] according to which these multiple mechanisms may be in fact related to the utilisation of drug concentration under varied experimental conditions. Nevertheless, two data seem to be formerly established: the first is that at drug concentration reflecting plasma concentration after bolus administration, the mechanism of drug action is likely to be through interaction with topoisomerase II by stabilisation of the ternary complex between DNA–topoisomerase II–drug [3]. The second is related to the

mechanism of the cumulative cardiotoxicity of anthracyclines leading to congestive heart failure [4, 5] with, consequently, maximum recommended cumulative DNR and DOX doses of 500 and 450–600 mg m<sup>-2</sup>, respectively. In addition to this factor which limits the optimal effectiveness of DNR and DXR, another limitation is the development of spontaneous and acquired resistance (vide infra). Therefore, intensive researches to find new analogues have been developed by either biosynthetic studies or syntheses. Biosynthetic studies have led to more than 300 new compounds [6] whereas more than 2000 analogues were issued from structural modifications of natural compounds or from total syntheses [7–9].

Among them, one can cite as marketed drugs carminomycin (or carubicin) (**3**), produced by a strain of *Actinomyces carminata*, originally developed [10] in the Soviet Union in the 1980s, Aclacinomycin A (or aclarubicin) (**4**), the only class II anthracycline [11, 12] to have been once marketed both in France and Japan for the treatment of leukaemia, daunorubicin benzoylhydrazone

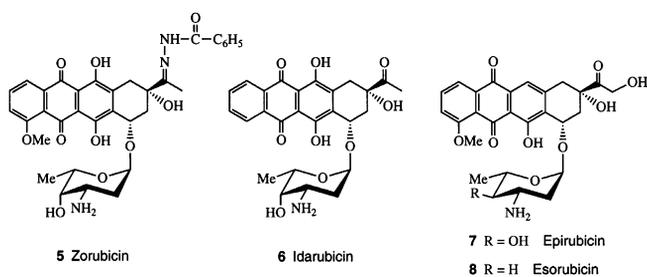


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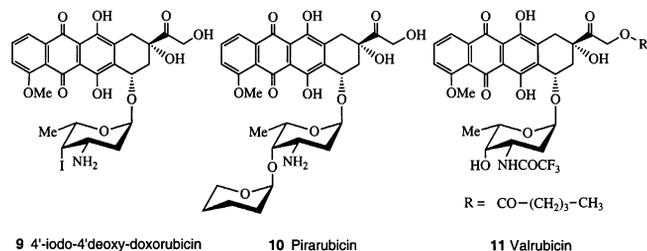
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or zorubicin or Rubidazone<sup>®</sup> (**5**) [13], idarubicin or 4-demethoxy-DNR (Zavedos<sup>®</sup>) (**6**) [14], and epirubicin or 4'-*epi*-DOX (Farmorubicin<sup>®</sup>) (**7**) [15].

Esorubicin (**8**) and 4'-iodo-4'-deoxy-doxorubicin (**9**) have not been developed beyond phase II trials, although they exhibited preclinical activity similar or superior to DOX on colorectal models and metastatic breast cancers, respectively [16, 17].



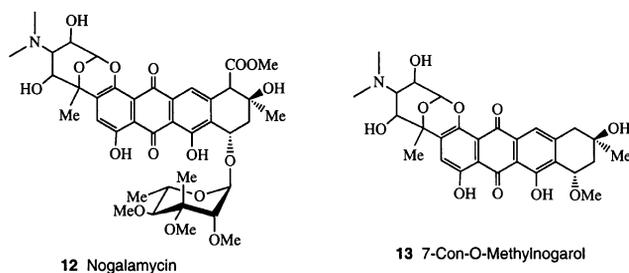
Pirarubicin, a 4'-tetrahydropyranyl-doxorubicin (**10**), was synthesised and developed in Japan [18], and subsequently marketed in several European countries including France (Theprubicin<sup>®</sup>). Evidence suggested that this compound acts as a primary drug and not as a prodrug [19].



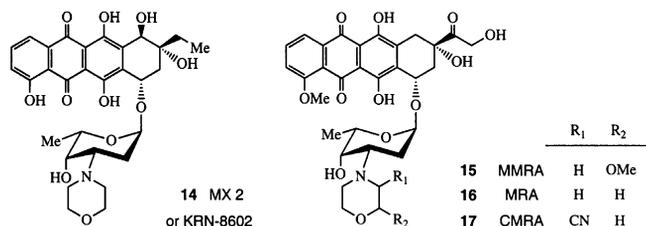
Valrubicin, or AD-32 (**11**), which was recently marketed under the name of Valstar<sup>®</sup> [20], is indicated for intravesical therapy of BCG-refractory carcinomas in situ of the urinary bladder. Clinical trials are currently in progress for the treatment of papillary cancers of the bladder.

Nogalamycin (**12**) which was isolated and characterised by Wiley et al. in 1968, was subsequently modified [21, 22] to give access to menogaril, also referred as 7-con-*O*-methylnogarol or TUT-7 (**13**), a drug developed by the Upjohn Company. Menogaril differs from DOX in several respects: the amino-sugar is attached to the D-ring through a glycosidic linkage and a C–C bond whereas, at the C-7 position, daunosamine is replaced

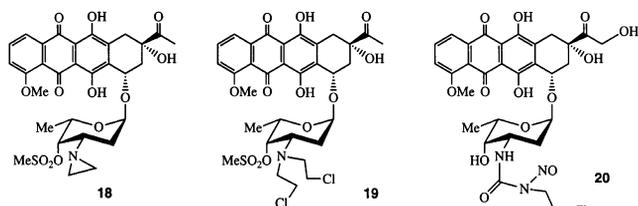
by an OMe group. Displaying greater activity, and lacking the acute toxicity which prevents development of nogalamycin, menogaril has been selected for clinical trials. Phase I clinical studies were conducted in Japan on malignant lymphomas [23, 24] and, in the United States, in patients previously treated for acute leukaemia [25, 26], as well as for treatment of recurrent astrocytomas in adults [27]. It is interesting to note that nogalamycin poisons topoisomerase I but not topoisomerase II, whereas the situation is opposite with menogaril. Such a differential poisoning of topoisomerases seems to be dictated by the minor groove binding nogalose sugar [28].



Among the series of anthracyclines of third generation, morpholinyl anthracyclines which have been the subject of many investigations constitute an interesting class of compounds, being active in vitro and in vivo against MDR tumours and altered topoisomerase MDR cells. Two molecules of this class are currently being investigated in phase II clinical trials, i.e. KRN 8602 (or MX2) (**14**), developed in Japan by Umezawa's group since the 1980s [29], and FCE 23762, or MMRA, a 2(*S*)methoxy-4-morpholinyl doxorubicin (**15**) developed by Pharmacia [30] in Italy. This compound is about 80 times more potent than doxorubicin and presents equivalent antitumour activity when administered by i.p., i.v. or by the oral route. Morpholino doxorubicin (or MRA) (**16**) and cyano-morpholino doxorubicin (or CMRA) (**17**) which are closely related to MMRA, were also prepared [31]. All these morpholino derivatives were ca. 100-fold more polar than classical anthracyclines, and they were claimed to be non-cardiotoxic at therapeutic levels. They have distinct mechanisms of action, since CMRA causes DNA cross-link, whereas MRA and MMRA act through topoisomerase I inhibition and DNA cross-linking.



Few years ago, a group of alkylcyclines which represent a new class of molecules was reported, with both DNA alkylating and intercalation properties. PNU-159548 or FCE 28729 (**18**) [32], a 4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin, is the lead compound of this novel series. This molecule is endowed with a double mechanism of action: DNA intercalation via the anthracycline backbone, and DNA covalent binding via the reactive alkylating group on the sugar moiety. Selected among other derivatives such as PNU-159550 (**19**), PNU-159548 displays high antitumour activity in a number of preclinical tests and a favourable toxicity profile [33]. It is also able to circumvent the resistance to all major classes of cytotoxics including MDR-related drugs, alkylating agents and topoisomerase II inhibitors. Due to its high lipophilicity, PNU-159548 (**18**) may cross the blood–brain barrier, and is effective against intracranial tumours, and the dose-limiting toxicity of this compound is myelosuppression whereas lack of cardiotoxicity may be explained, at least partially, by high plasma clearance [34]. Phase II clinical trials of this drug are underway.

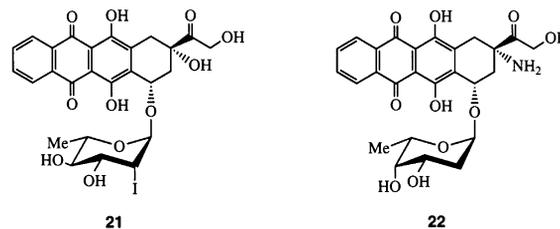


A new compound of this class of alkylcyclines is the metabolite of methoxy-morpholino-DOX, which displays impressive potency *in vitro* ( $IC_{50} = 0.0021$  ng mL<sup>-1</sup>) against L1210. The synthesis of this compound and its biological properties has recently been reported [35].

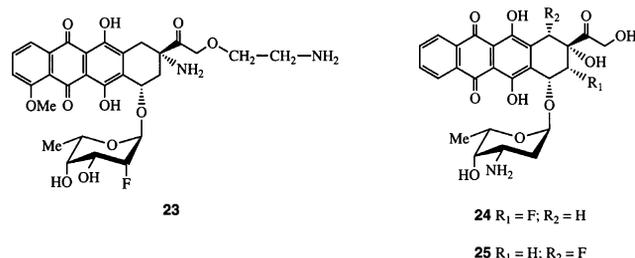
Based upon the same concept hybrid, 2-chloroethylnitrosoureido-anthracyclines derived from DOX (AD-312, **20**), DAU (AD-347), and epirubicin (AD-392) have

been synthesised. AD-312 circumvents resistance conferred by either reduced DNA–topoisomerase II or increased P. glycoprotein overexpression. Thus cellular resistance to this hybrid should be due partially to an O-6-methylguanine–DNA methyltransferase activity [36].

Priebe and Perez-Soler explored [37, 38] a 'double advantage approach' for designing more effective anticancer anthracyclines able to overcome MDR substitution. Anthracycline antibiotics with reduced basicity and increased stability of the glycosidic bond were thus synthesised. So far, among the numerous compounds which have been prepared, 2'-iodo-3'-deamino-4-demethoxy-DOX or Anamycin (**21**) appears to be the most promising compound. Anamycin proved to be non-cross-resistant, or partially cross-resistant with DOX, while displaying high antitumour activity. Therefore, Anamycin has been incorporated in a liposomal formulation for phase I clinical study and pharmacological study [37, 38].



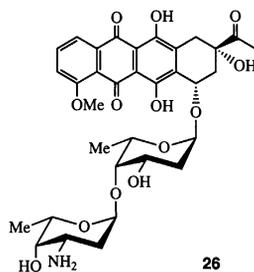
Amrubicin hydrochloride (**22**), a glycoside of synthetic 4-demethoxy-9-deoxy-9-amino-daunomycinone [39] is currently under phase II study for effective treatment of advanced non-small cell lung cancer, whereas DA-125 (**23**) is presently developed by Dong Pharma and tested against both human gastric and pulmonary adenocarcinoma cells resistant to DOX [40].



Taking into account the fact that anthracyclines act primarily by poisoning topoisomerase II through a blockage of the DNA religation step 8- and 10-fluoro derivatives were synthesised by Menarini's group [41],

both in the DNR series and in the 4-demethoxy series. 8(*R*)-Fluoro-idarubicin (**24**) was the most effective in stimulating topoisomerase II-mediated DNA cleavage, and was almost as effective as DOX in the inhibition of human ovarian carcinoma xenografted in nude mice. The 10-fluoro derivatives were less potent than the parent drugs although 10(*S*)-fluoro-idarubicin (**25**) inhibited by 50% the growth of all 58 cell lines tested by the NCI.

The same group also reported [42] the synthesis of new derivatives of anthracycline disaccharides, the design of which was based on several considerations, among which the displacement of the amino group of daunorubicin to the second sugar moiety should have a consequence in both the sequence specificity and in the recognition of protein domains in the minor groove. Among different analogues, the 2-deoxyfucosyl-daunosaminyl-glycoside of 4-demethoxy-DNR (**26**) [43] showed high efficacy in the inhibition of a spectrum of human tumours xenografted in nude mice, and is presently entering phase I clinical trials under the auspices of EORTC [44].



During the last decade, drug carrier technology has been intensively developed with the aim of overcoming MDR, or to reduce the cardiotoxicity of anthracyclines. As reported by Perez-Soler et al. [45], the pharmacological methods for targeting anthracyclines to tumours may be classified into two main groups, depending on whether they imply a specific recognition of the tumour by the carrier or simply a preferential drug distribution.

Compared to microspheres or nanoparticles, liposomal incorporation of drugs represents the leading method to passively target anthracyclines to tumours.

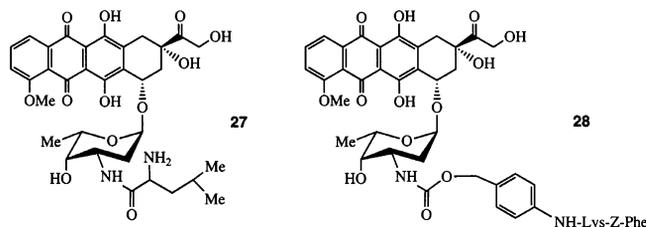
Encapsulation of DNR and DOX in liposomes was first studied ten years ago with the aim of modifying the pharmacokinetics of such anthracyclines with increasing drug selectivity. Three main liposomal formulations have been marketed: Lipodox<sup>®</sup> or TLC D-99 developed by Pfizer [46], Doxil<sup>®</sup> or Caelyx<sup>®</sup> marketed by Sequus

Pharmaceuticals [47] and DaunoXome<sup>®</sup> from Nexstar [48] which was tested with promising results in patients with AIDS-related Kaposi's sarcomas [49]. Safety and potential usefulness of such liposomes have been recently reported in patients with advanced recurrent malignant CNR tumours [50]. Less successful is the use of such liposomal formulations in other solid tumours, as indicated by some phase II trials with DaunoXome [51] or in melanoma [52] and soft tissue sarcomas with doxil [53].

Selective targeting of immunoliposomal DOX has been addressed against myeloma by using anti-CD19 antigens [54] or by incorporating phosphatidylethanolamine lipids containing hyaluronan oligosaccharides derivatives, which avidly bind to CD44 expressing tissues such as various carcinoma, melanoma and other tumours [55]. On the other hand, coupling transferin to the distal ends of liposomal polyethylene glycol (PEG) chains led to significant increased glioma DOX uptake compared to other liposome populations [56].

Numerous polymers have been prepared to entrap anticancer drugs for subsequent selective release in target tumours. Postulated mechanisms involve a transportation of the drug-carrier conjugate and further lysosomal hydrolysis to release the cytotoxic principle. Among the first attempt with anthracyclines, are the conjugates daunorubicin and doxorubicin-DNA [57, 58] but no advantage over free DOX was found in the only clinical trial which was conducted [59].

Among different conjugates of anthracyclines to aminoacids resulting from further developments of the 'lysosomotropic theory' by Trouet's group, *N*-L-leucyl-doxorubicin (**27**) emerged as the most promising candidate, and it was selected for clinical evaluation by EORTC [60]. Acting as a prodrug, *N*-L-leucyl-doxorubicin is less toxic than DOX, and its superior efficacy versus DOX in human melanoma xenografts has been recently reported [61, 62] to correlate with high tumour concentrations of free drug.

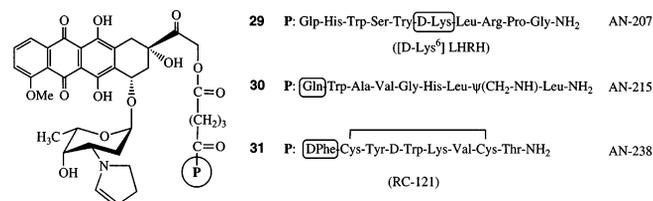


Found in relatively high levels in mammalian lysosomes and expressed in the extracellular medium in metastatic or primary tumours, cathepsin B was designed as a target. The cathepsin B-sensitive dipeptide prodrug of DOX, which includes a self-immolative spacer, was thus prepared [63, 64] as a model compound (28) for internalising anticancer immunoconjugates.

Based upon the same idea that many cancers exhibit elevated levels of peptide hormone receptors, several series of peptide hormone–anthracycline conjugates have also been designed for the last two years such as macromolecular carbomethoxypullulan–peptide–doxorubicin conjugates [65] leading to a selective release of DOX *in vivo*, and membrane-active cationic peptide–doxorubicin conjugates [66]. Notably, MCF-7/ADR resistant cells were actually somewhat more sensitive to these cationic peptides than the parental MCF-7 cells. Both kinds of peptides enhance the cytotoxic effect of DOX.

Doxorubicin and 2-pyrrolidino-DOX were also linked to different kinds of peptides by a glutaric acid spacer. A first series includes analogues of luteinising hormone-releasing-hormone (LH-RH) such as AN-207 (29), which binds to receptors present on membranes of various tumours [67]. The mechanism of action has been demonstrated using fluorescent AN-152, the corresponding DOX-conjugate of AN-207. Nuclear subcellular localisation of a two-photon fluorophore-linked version of AN-152 has been outlined [68] as well as was displayed the regulation targeted chemotherapy of this conjugate by EGF [69].

The rationale for the synthesis of bombesin-like peptides containing DOX or 2-pyrrolidino-DOX proceeds from extensive evidence that peptides of the tetradecapeptide bombesin family (BN) are involved in various tumour cells [70]. Peptide fragments of the C-terminal sequence of bombesin were thus conjugated to DOX through the 14-*O*-hemiglutarate intermediate to afford a series of conjugates such as BN analogue AN-215 (30). These conjugates were further converted to 2-pyrrolidino-DOX-containing analogues. *In vivo* studies clearly

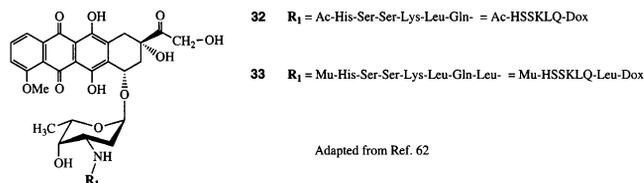


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demonstrated that activation and internalisation of the prodrug is dependent on binding to the bombesin receptor.

Cytotoxic analogues of somatostatin containing doxorubicin or 2-pyrrolidinodoxorubicin have been prepared by the same group [71] and tested on various human cancer cell lines. These highly active cytotoxic conjugates have been designed to target cancer expressing receptors for somatostatin octapeptides and preliminary studies in animal models showed that some of them, cf. AN-238 (31), are able to inhibit tumour growth [72]. Efficacy was observed only in human tumours that express somatostatin receptors [73, 74]. Thus, a positive answer was obtained against human glioblastoma cell line xenografts with AN-238 whereas 2-pyrrolidino-DOX had no significant effects. In order to improve the efficacy of these prodrugs, addition of diisopropyl fluorophosphate as serum carboxylesterase enzymes has been achieved. Significant increase of the half-life of such prodrugs and three-fold increase in the maximum tolerable dose were observed when such a carboxylesterase inhibitor was co-administered *in vivo* in nude mice [75].

Protease-activated conjugates represent another class of new compounds. Indeed, in order to target DOX to metastatic prostate cancer, coupling of the amino group of DOX was done to give conjugates 32 and 33 which are selectively hydrolysable by the serine protease prostate-specific antigen (PSA) and thus afford L-leucyl-DOX [76]. Much less cytotoxic than DOX to cells in culture that do not secrete PSA, L-377,202, another conjugate (*N*-glutaryl-[4-hydroxypropyl]-Ala-Ser-cyclohexaglycyl-Glu-Ser-Leu-DOX) was approximately 15 times more effective than DOX at inhibiting the growth of human

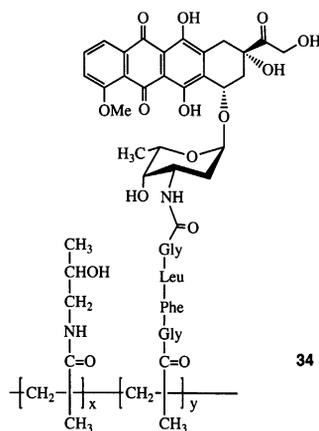


prostate cancer tumours in mice [77]. Moreover, unlike DOX, L-377,202 did not induce cardiotoxicity in dogs.

Very recently it has also been reported that vectorisation of DOX with specific peptide vectors reduces significantly its accumulation in the heart [78] but also that

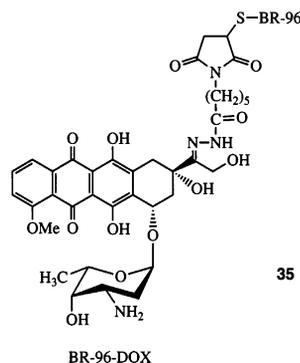
such DOX–peptide conjugates may overcome multidrug resistance when tested in human erythroleukaemic (K562/ADR) resistant cell lines [79].

At the beginning of the 1990s, Duncan and co-workers [80] developed PK 1 (**34**), a polymeric molecule containing doxorubicin (about 8.5% w/w) of MW 24 000. This conjugate consists on a [*N*-2(hydroxy-propyl)methylacrylamide] polymer bound to DOX through its 3'-amino group via an oligopeptide spacer. The rational design of this system was based on a preferential capture of PK 1 by tumour tissues, and subsequent local release of DOX through a proteolytic degradation. Polymer PR 1 exhibits reduced general toxicity. It is highly active against a number of murine tumour models, both DOX-sensitive and DOX-resistant [81], being especially active in solid tumour models. Pharmacokinetic studies of PK 1 have shown [82] that the level of DOX is low but persistent after its i.v. administration, whereas, after i.v. administration of DOX, the plasma level is high but decreases rapidly. All these data warrant further evaluation in humans by Pharmacia Upjohn (Italy).



Immunotargeting tumours with DOX conjugated to antibodies has been considered as an attractive strategy, and several conjugation technologies have been utilised to link DOX or DNR to monoclonal antibodies. The most studied derivatives belong to the class of disulfide conjugates of hydrazone derivatives of DNR and DOX, and the monoclonal antibodies were successively MAb BR-64 and MAb BR-96, which is more tumour-selective. These two MAb identify a Lewis-Y-related tumour-associated antigen expressed at high levels [83, 84]. Although these immunoconjugates, especially BR-96 DOX (**35**), are significantly more potent and efficient

than the parent drug, many problems remain to be solved, such as better stability [85] or increasing the DOX/MAB molar ratio without significant losses in antigen-specific binding [86]. Although a DOX/MAB molar ratio of 30–40 was obtained when the drug was coupled to aminodextran, as in Gentran 40 [87], the therapeutic efficacy was still limited. Some recent syntheses have been proposed, such as that which consists in enzymatic assembling of DOX on galactose residues [88]. Among the multitude of problems inherent to this approach are the heterogeneity of antigen expression, and the fact that once the conjugate binds to the tumours cell, it must be internalised to exert its effect.

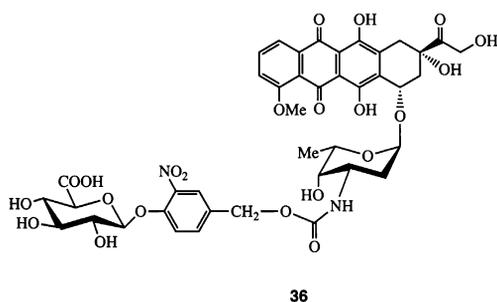


This internalisation is crucial and the vast majority of tumour-associated antigen, and thus antibody-drugs, are not internalised. Those conjugates that are internalised are often transported to the lysosome of the cell where the drug is degraded [89].

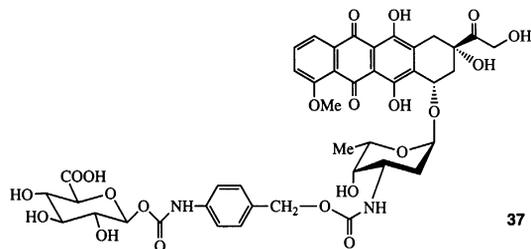
An alternative strategy to overcome most of the problems of selectivity and potency was proposed [90, 91] at the end of the 1980s under the name 'A.D.E.P.T.' for Antibody Directed Enzyme Prodrug Therapy. This concept aims at using antibodies as vectors for enzymes capable of activating a non-toxic drug, termed a 'pro-drug' to a potent cytotoxic drug. Thus, in the first phase, a tumour-specific antibody enzyme conjugate is administered. After an appropriate localisation time (generally 8–10 days), the conjugate which is not specifically bound to the antigens of the tumour cells will have been cleared off from the blood and normal tissues. In the second phase, a non-toxic prodrug suited for activation by the targeted enzyme is injected.

Among the various prodrugs of DNR and DXR which have been elaborated [92–94], HMR 1826 (**36**), a glucuronide-based prodrug of DOX, emerged as the

most promising for clinical applications [95]. This may be explained by several factors: the use of a fusion protein well defined, including a monoclonal antibody anti-CEA (Mab) and the  $\beta$ -glucuronidase [96], instead of a conjugate; an expected low immunogenicity due to the fact that the Mab is humanised and the enzyme is of human origin; the access to this three-component prodrug **36**, efficiently hydrolysed and well detoxified [97, 98]. Indeed higher therapeutic efficacy and lower cardiotoxicity of HMR1826 versus DOX were clearly established [99].



Human  $\beta$ -D-glucuronidase was subsequently investigated by the group of Haisma [100–102] in the Netherlands to activate the prodrug **37**, closely related to HMR-1826. Subsequently they constructed fusion proteins consisting of either single chain anti-CD20 antibody [103], or single chain anticarcinoma antibody 323/A3 [104] with human  $\beta$ -glucuronidase.



Prodrug therapy based on elevated tumour levels of  $\beta$ -glucuronidase in necrotic area has been proposed by Bosslet et al. [105] because both approaches can utilise the same glucuronide prodrugs. Elevated activity of  $\beta$ -glucuronidase in tumour tissues was first observed by Fishman [106] 40 years ago, but utilisation of this increased tumour activity in selective application of prodrugs has not been widely employed [107, 108]. Validation of this renewed concept was clearly demon-

strated by the ex vivo experiment conducted by Mürdter et al. [109] on a perfused human lung model with HMR-1826 and in pancreatic cancer [110]. A recent comparison of three approaches of DOX therapy using free DOX, liposomal DOX and HMR 1826 led to the conclusion that the  $\beta$ -glucuronidase activated prodrug is safe and effective [111].

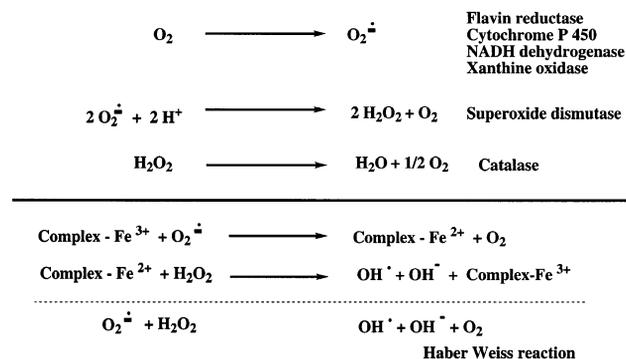
Another strategy to selectively deliver oncostatic drugs was proposed two years ago. In vivo, selection of phage display libraries was used by Ruoslahti et al. [112] to isolate peptides that bind specifically to tumour blood vessels. The results of their study imply that many tissues have vascular addresses. Recovery of phage from the tumours led to the identification of three main peptide motifs that targeted the phage into the tumours. When coupled to doxorubicin, two of these peptides, one containing an  $\alpha_v$ -integrin-binding motif, the Arg-Gly-Asp (RGD) and the other an Asn-Gly-Arg (NGR) motif, enhanced the efficacy of the drug against human breast cancer xenografts in nude mice and also reduced the toxicity. Particularly interesting is this new targeting strategy, since the tumour vasculature is composed of non-malignant endothelial cells that are genetically stable, and therefore unlikely to mutate into drug-resistant strains. Other advantages lie in the fact that endothelial cells are more accessible to drugs and have an intrinsic amplification mechanism.

The two main problems, cumulative cardiotoxicity and acquired resistance, which limit the clinical usefulness of anthracyclines, have received much attention during the past few years.

Cardiotoxicity due to the cumulative effect of DOX is a dose-limiting effect [113] and certain factors have been identified, which favour the appearance of anthracycline-induced cardiotoxicity such as the age and the existence of a family background of cardiovascular deficiency. Other factors which can be involved are the association with other antimetabolites, in particular with cyclophosphamide and with taxanes, widely associated in the treatment of metastatic breast cancer or dose escalation in clinical treatment (epirubicin for instance).

The mechanisms through which DOX generates cardiotoxicity are multifactorial and complex [114]. It is widely admitted that anthracyclines can be reduced to semi-quinone derivatives of radical nature in the presence of enzymes such as NADPH-cytochrome P450 reductase or NADH dehydrogenase. As a consequence, transfer of one electron to molecular oxygen leads to

the formation of a superoxide radical, which, in turn, may give hydrogen peroxide in the presence of catalase or hydroxyl radical according to the Haber–Weiss reaction, catalysed by  $\text{Fe}^{3+}$ . Usually, the superoxide radical is neutralised by superoxide dismutase, and the resulting hydrogen peroxide is, in turn, decomposed to water and molecular oxygen by catalases, or reduced to  $\text{H}_2\text{O}$  by glutathion peroxidase. In comparison with other tissues, the heart is poor in catalase, and glutathion peroxidases are destroyed by anthracyclines. As a result, hydrogen peroxide accumulates, thus leading, by reaction with oxygen superoxide, to an increased formation of OH radical which may produce acute destruction of mitochondria, endoplasmic reticulum and nucleic acids.



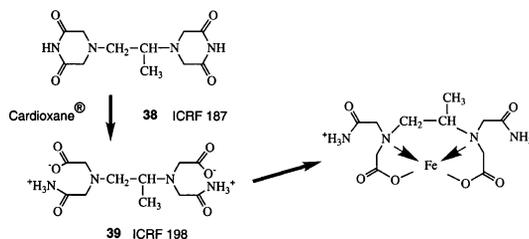
Based upon these data, a large number of radical scavengers have been tested without great success until the role that ferric ion plays in the mechanism of OH formation (Fenton reaction) was understood [115].

Dextrazoxane (ICRF 187, **38**), a member of the bis-dioxopiperazine family, was initially tested as an anti-cancer drug both in animal models and in clinical trials. It was eventually found to have a cardioprotective property [116]. The active form is the pure *S*-enantiomer synthesised by a stereoselective process [117].

Structurally, it is a lipophilic derivative of EDTA, a well-known chelating agent of  $\text{Ca}^{2+}$  and heavy metal metabolism. After entering the cells, ICRF 187 (**38**) is metabolised to ICRF 198 (**39**), which is able to trap  $\text{Fe}^{3+}$  and therefore inhibit the Haber–Weiss reaction.

Phase II studies have provided information on the tolerance of this product in monotherapy without interference with the antitumour efficacy of anthracyclines. The benefit of the cardioprotection conferred by ICRF

187 has been observed during phase II studies [118, 119].



Resistance to anthracycline treatments remains a tremendous problem for clinicians [120]. Although there were a large number of modulators of MDR, some of them being of high efficacy *in vitro* and *in vivo* on animal models [121], the attempts to improve therapeutic response in patients were limited due to intrinsic toxicity.

Besides the identification of the Pgp as responsible for multidrug resistance (MDR), another ABC transporter, the multidrug associated protein (MRP1) has been further identified. Like Pgp, MRP1 is a membrane-associated protein which confers resistance to the anthracyclines [122–124]. The search for MRP-1 modulators has led to the identification of a series of compounds which have been recently reviewed [125]. However, the finding of more effective agents is still a need. Other mechanisms for explaining the anthracycline multidrug resistance have been postulated such as the glutathion conjugation of the drug which led to its retention in the cytoplasm, predominantly in the golgi region [126]. From there, glutathion peptidomimetic drug has been designed as an effective inhibitor of MRP-1 mediated resistance [127].

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