Hydrazone, Amide, Carbamate, Macromolecular and Other Prodrugs of Doxorubicin

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Abstract: An important strategy for improving the antitumor selectivity and decreasing severe side effect of antitumor agents is to design carrier systems and prodrugs. Here, we review the most important prodrug models based on doxorubicin.

Keywords: Doxorubicin, prodrug, hydrazone, amide, carbamate, macromolecular.

1. INTRODUCTION

The term prodrug was first used by Albert [1]. Prodrug can be defined as an agent that is transformed after administration, either by metabolism or by spontaneous chemical breakdown, to form a pharmacologically active drug. The prodrug itself is inactive or less active and is converted to active agent *in vivo*. Targeted prodrug approach is one of the new trends in the treatment of cancer. A lot of antitumor drugs possess a limited bioavailability due to low chemical stability, limited oral absorption or rapid metabolism [2]. Because of these problems, different prodrug models that can be activated into antitumor drugs have been designed. An improved solubility can facilitate oral absorption, while improving chemical stability of an active drug. An ideal prodrug is designed to increase the bioavailability and to eliminate undesirable side effects of antitumor drug. Prodrugs of antitumor agents are also designed as organ-specific and tumor-specific targeting. An important strategy to achieve local activation of prodrugs is the use of enzyme immunoconjugates. In this approach, which is called antibody-directed enzyme prodrug therapy (ADEPT) [3], an enzyme is conjugated to a tumor-specific antibody. Alternative approaches for the design of ADEPT are gene-directed enzyme prodrug therapy (GDEPT) [4-6] and virus-directed enzyme prodrug therapy (VDEPT) [7].

Doxorubicin (DOX) (Fig. **1**) or hydroxyldaunorubicin are antracycline antibiotics and commonly used in the treatment of a wide range of cancers such as Hodgkin's disease, non-Hodgkin's lymphomas, acute leukaemia, bone and soft-tissue sarcoma, neuroblastoma and malignant neoplasms of the bladder, breast, lung, ovarium and thyroid [8]. DOX is known to interact with DNA by intercalation and inhibition of macromolecular biosynthesis. This drug inhibits the progression of the DNA polymerase and topoisomerase II. In addition, DOX can directly bind iron. The amount of iron (Fe) in the cell is regulated by the iron regulatory proteins (IRPs)-1 and -2. Although, DOX is an effective anticancer agent, its use is limited by cardiotoxicity, an effect associated with their ability to chelate Fe and perturb Fe metabolism. Despite its clinical efficacy, DOX suffers from drawbacks that are common for all chemotherapeutic agents: it is not tumor selective therefore affects healthy tissue causing severe side effets such as cardiotoxicity [9], and intrinsic or acquired resistance to the drug that is developed by tumors [10]. To minimize the dose-related toxic side effects of DOX, by using endogenous and exogenous enzymes, various prodrug designs have been developed, especially for delivering the drug to the tumor cells as targeted.

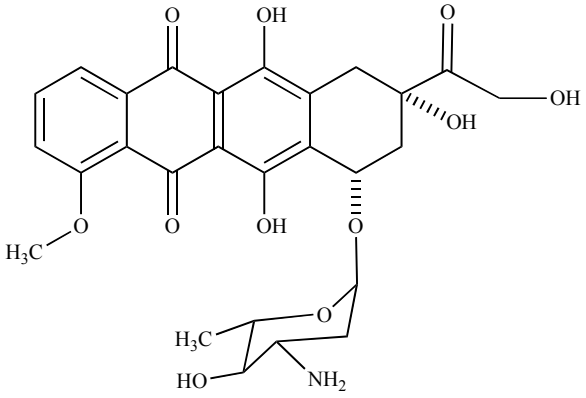

Fig. (1).

2. PRODRUGS

2.1. Hydrazone and Amide Prodrugs of Doxorubicin

Hydrazones constitute an important class of compounds for new drug development. Therefore, many researchers synthesized these compounds as target structures and evaluated their biological activities (for see review [11]). Hydrazones can be hydrolysed to their coresponding carbonyl compounds. Several studies have been reported about the *in vitro* and *in vivo* metabolism of hydrazide-hydrazones [12-15]. The formation of hydrazone and amide is one of the useful methods for prodrug synthesis due to convertion of active drug by hydrolysis.

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DOX-hydrazone has been linked to a thiol-containing monoclonal antibodies (mAb) to produce its immunoconjugates. DOX-hydrazone-immunoconjugates are internalized into lysosomes with acidic character. Therefore DOX is released by hydrolysis of the acid-labile hydrazone bond [16]. In 1993, Willner *et al*. [17] synthesized the (6-maleimidocaproyl)hydrazone of doxorubicin (MC-DOXHZN) as a new derivative for the preparation of immunoconjugates of doxorubicin. Conjugates were obtained from various mAbs, including chimeric BR96 (Fig. **2**). BR96-conjugates of DOX was tested under acidic conditions that mimic the lysosomal environment and showed antigen-specific cytotoxicity.

Kratz *et al*. [18] have developed DOX-hydrazones and DOX amides to increase the therapeutic index of DOX. Hydrazone derivatives of DOX were synthesized by reacting DOX with 3-maleimidobenzoic acid hydrazide (Tf-Hyd_1) or 4-maleimidophenylacetic acid hydrazide (Tf-Hyd_2) (Fig. **3**). The amide derivatives of DOX were synthesized by reacting DOX with 3-maleimidobenzoic acid (Tf-$Amid_1$) or 4-maleimidophenylacetic acid (Tf-$Amid_2$) (Fig. **4**). Thiolated human serum transferrin was conjugated with maleimide derivatives of DOX. They reported that the carboxylic acid hydrazones of DOX exhibited an inhibitory efficacy in the MDA-MB-468 breast cancer cell line and U937 leukemia cell line comparable to that of the free drug and amides had no antiproliferative activity in the MDA-MB-468 breast cancer cell line and U937 leukemia cell line.

The (6-maleimidocaproyl)hydrazone derivative of DOX (DOXO-EMCH) (Fig. **5**) which is defined as MC-DOXHZN by Willner *et al.* [17] is an albumin-binding prodrug of DOX with acid-sensitive properties. It is reported that DOXO-EMCH showed a good safety profile and was able to induce tumor regressions in tumor types known to be anthracycline-sensitive tumors [19].

Lactosaminated human albumin (L-HSA) is a hepatotropic drug carrier safely used in humans [20]. L-HAS coupled DOX has been increased DOX concentration in the tumors [21]. According to literature, L-HSA-DOX conjugate was synthesized using a (6-maleimidocaproyl)hydrazone derivative of DOX (DOXO-EMCH) [22, 23].

2.2. Carbamate Prodrugs of Doxorubicin

The carbamate prodrugs were designed for selective hydrolysis by human carboxylesterases to release active drugs. Glucuronylated prodrugs of DOX and derivatives have been developed and these prodrugs have carbamate moiety which obtained from glucuronic acid conjugate of DOX. They are less toxic and more polar than their parent drugs. *N*-[4-doxorubicin-*N*-carbonyl-(oxymethyl)-(4-nitrophenyl)]-*O*-β-glucuronyl carbamate (HMR 1826) [24] and *N*-[4-doxorubicin-*N*-carbonyl-(oxymethyl)-phenyl]-*O*-β-glucuronyl carbamate (DOX-GA3) [25] were synthesized and HMR 1826 and DOX-GA3 (Fig. **6**) were activated by human β-glucuronidase. DOX-GA3 was found to be stable upon incubation with human serum [26] and was shown to be even more effective than DOX [23]. DOX-GA3 was 12-fold less toxic than DOX in cells, in the human ovarian cancer cell line [27].

A disadvantage of hydrophilic glucuronide prodrugs is their elimination by the kidney [26]. The rapid elimination of

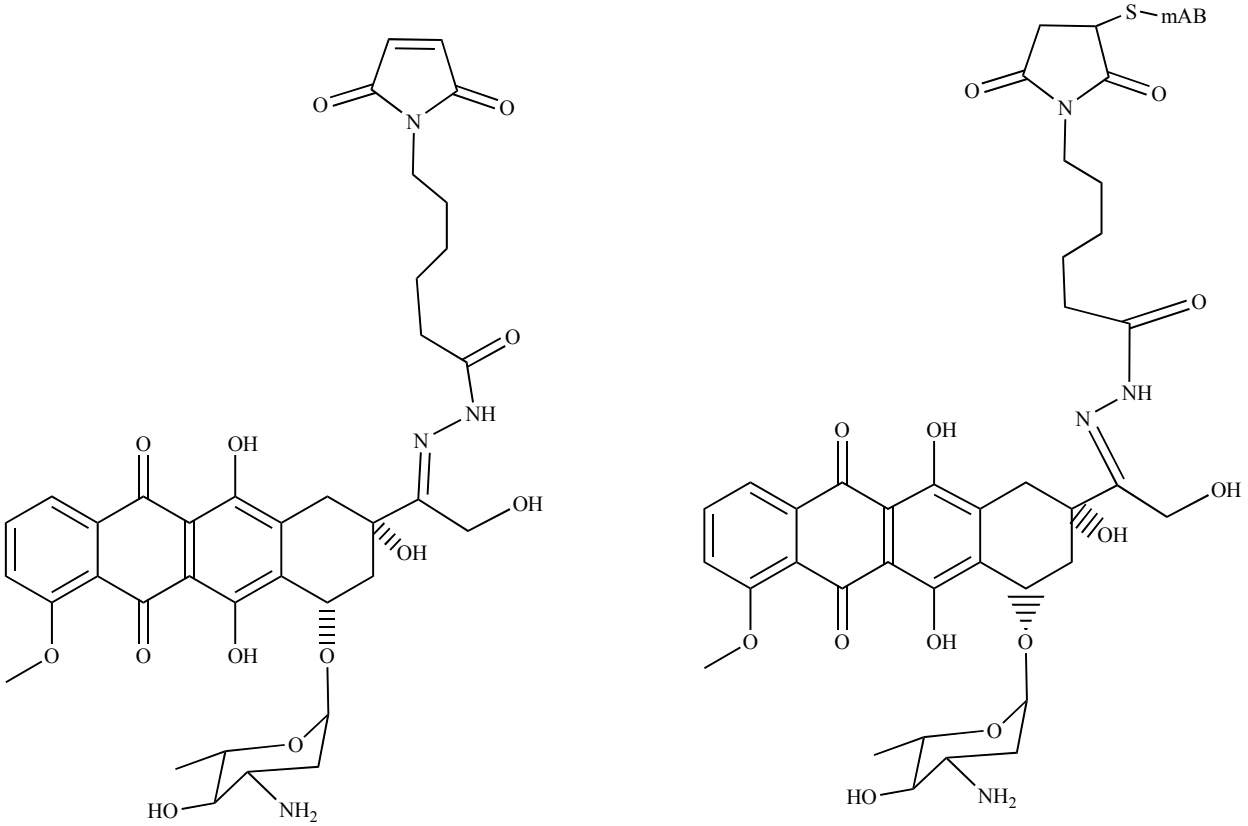


Fig. (2).

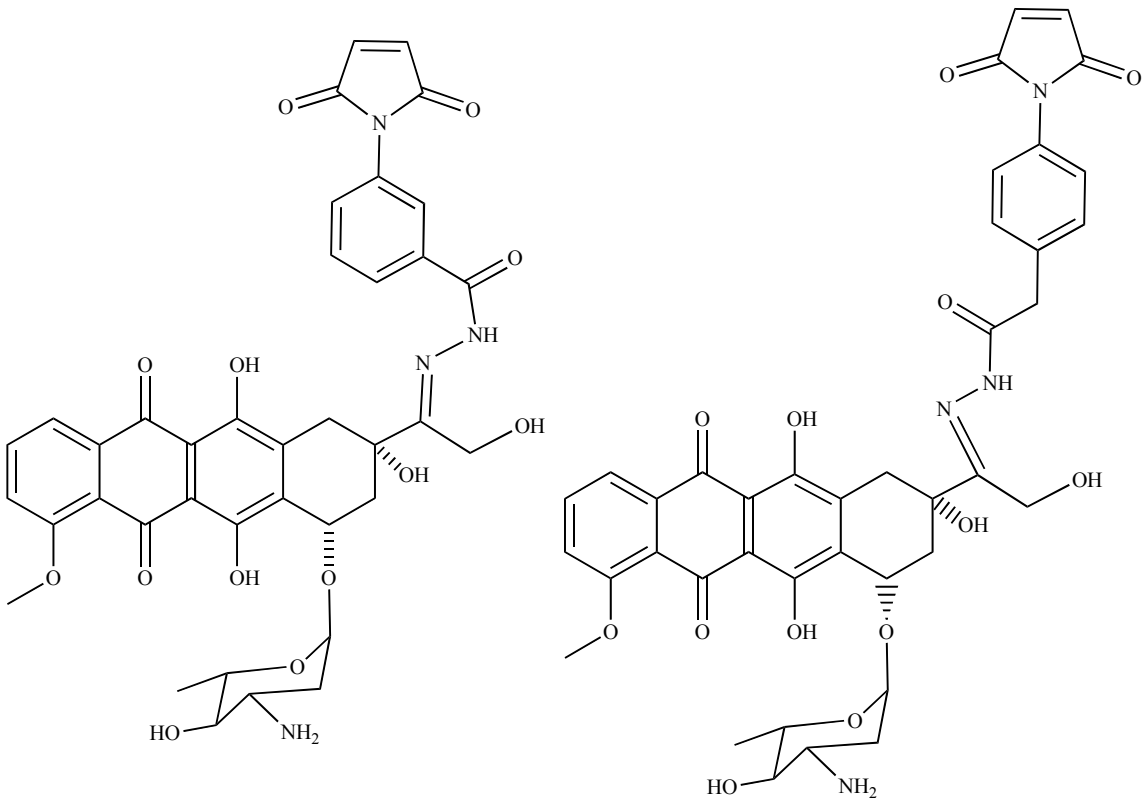


Fig. (3).

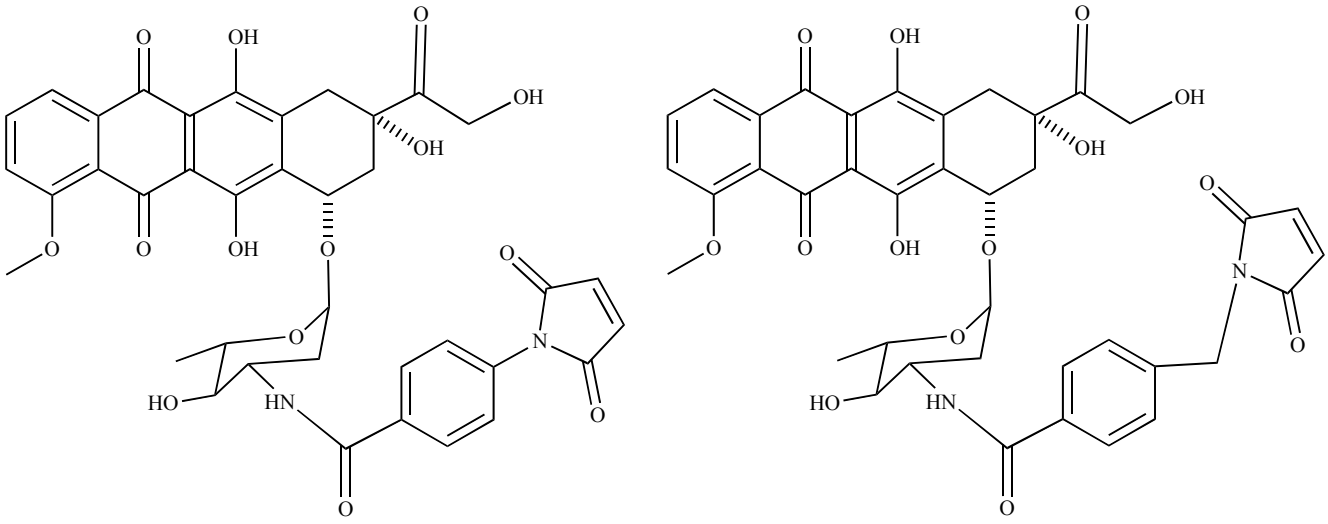


Fig. (4).

glucuronide prodrugs is a major problem for their use in cancer treatment, as very high doses are required. The ester of glucuronide prodrugs would be more lipophilic than glucuronide prodrugs. When administered *in vivo*, the ester of glucuronide prodrugs might be removed by carboxylesterase activity in plasma to yield the original glucuronide prodrug, which could be activated by human β-glucuronidase [28, 29]. Another disadvantage of glucuronide prodrugs, especially in case of DOX, is relatively inefficient synthesis [24, 25]. Graaf *et al*. [30] synthesized methyl ester (DOX-mGA3) of DOX-GA3 and reported pharmacokinetic properties of the lipophilic DOX-mGA3 as compared to that of the hidrophilic DOX-GA3.

Devalapally *et al*. [31] synthesized two new prodrugs (Fig. **7**) activated to doxorubicin by a lysosomal enzyme β-galactosidase. Activity of β-galactosidase in the breast and colon tumors is higher compared to the normal tissues. These prodrug that contain different spacers have carbamate moiety except a galactose moiety and DOX. They proposed *N*-[β-D-galactopyranosylbenzyloxycarbonyl)doxorubicin that was readily hydrolyzed as a valuable prodrug for further development.

Yoneda *et al*. [33] reported the synthesis of, a cyclic 13-mer oligopeptide, Pep42-doxorubicin prodrug conjugate containing a cathepsin B – cleavable linker. Pep42 that binds

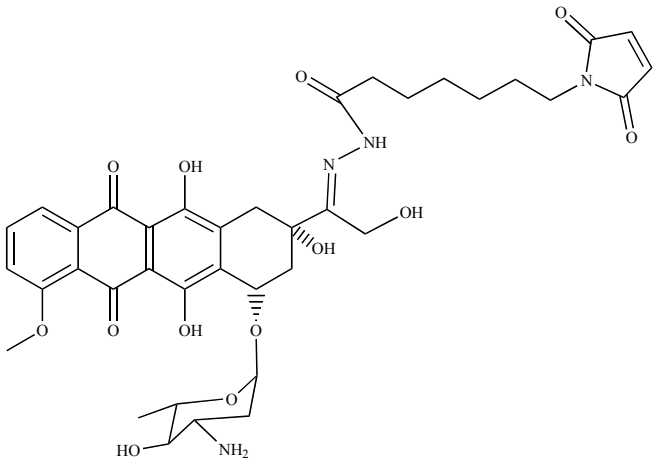


Fig. (5).

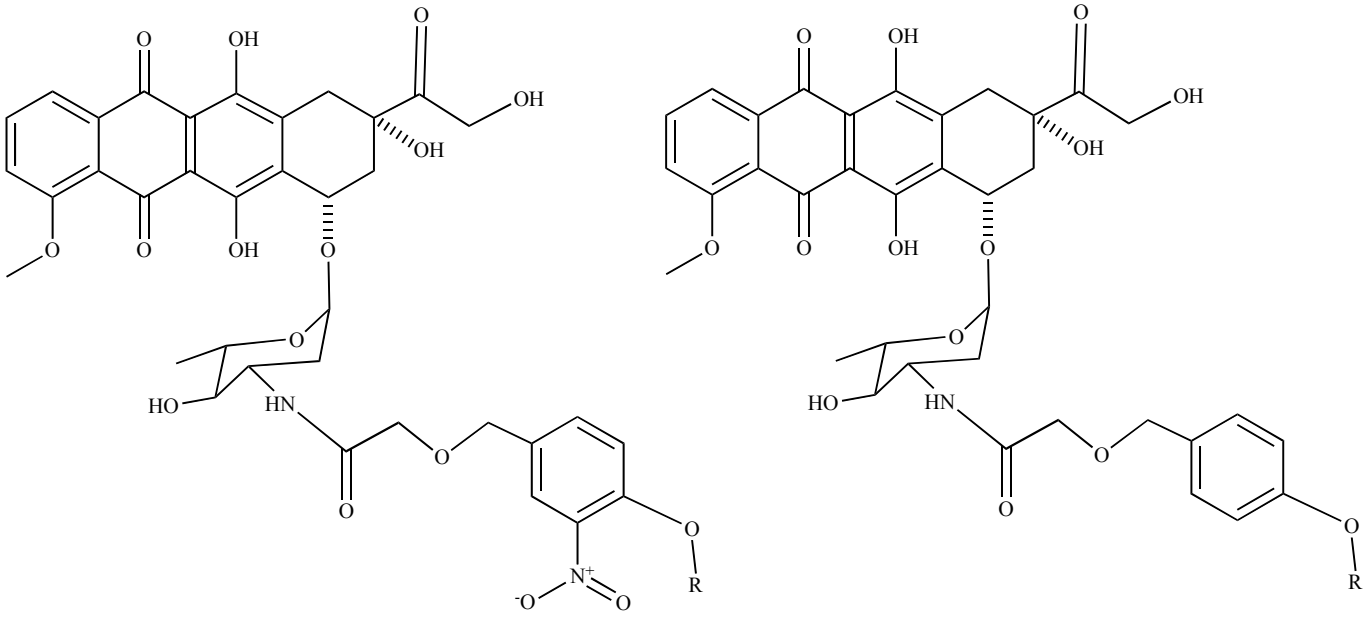


Fig. (6).

to glucose regulated protein 78 has linked to p-aminobenzyl alcohol that a commonly used self immunolative spacer has been attached to doxorubicin. Release cascade of the drug is represented. Bearing in mind doxorubicin-Pep42 conjugate's demonstration of enhanced cytotoxic activity against SJSA-1 cell and by employing the same activation release cascade new prodrug conjugates carrying tumor targetting peptides that release the drugs upon entering cells could frequently be used in current cancer therapy treatment regimes.

Hay *et al*. [32] reported the synthesis of DOX prodrugs containing nitrobenzyl– and nitroimidazoylmethyl carbamate moiety (Fig. **8**) and evaluated them for their potential use in nitroreductase mediated GDEPT.

2.3. Macromolecular Prodrugs of Doxorubicin

Polymeric prodrugs are the polymeric conjugates of conventional drugs and most of the polymeric prodrugs have been developed for the delivery of anticancer agents [34]. The synthetic polymer-anticancer drug conjugates is a promising approach to improve the efficacy, increase solubility and reduce the side effects of these drugs. Kratz *et al*. [35] demonstrated the endothelial layer of blood vessels in tumor tissue is often leaky. Therefore large molecules can be internalized by malignat tissue thus macromolecules can accumulate in solid tumor. Dendritic polymers have been widely developed as drug carrier and possess a large buffering capacity for carrying a lot of secondary and tertiary nitrogens [36]. Lai *et al*. [37] reported that the pH-activated polymer has been denoted as a succesful drug delivery vehicle system. DOX have been conjugated to the PAMAM (polyamidoamine) dendrimers as PAMAM-amide-DOX or PAMAM-hyd-DOX [36].

The water-soluble cyclotriphosphazene-DOX conjugate (Fig. **9**) was synthesized and it exhibited lower cytotoxicity than that of free DOX against the leukemia L1210 cell line [38].

The albumin doxorubicin conjugate was synthesized by coupling a maleimide carboxylic hydrazone derivative of doxorubicin, with thiolated albumin by the help of the HS group of cysteine-34 of human serum albumin which is the most reactive thiol group in human plasma [39, 40].

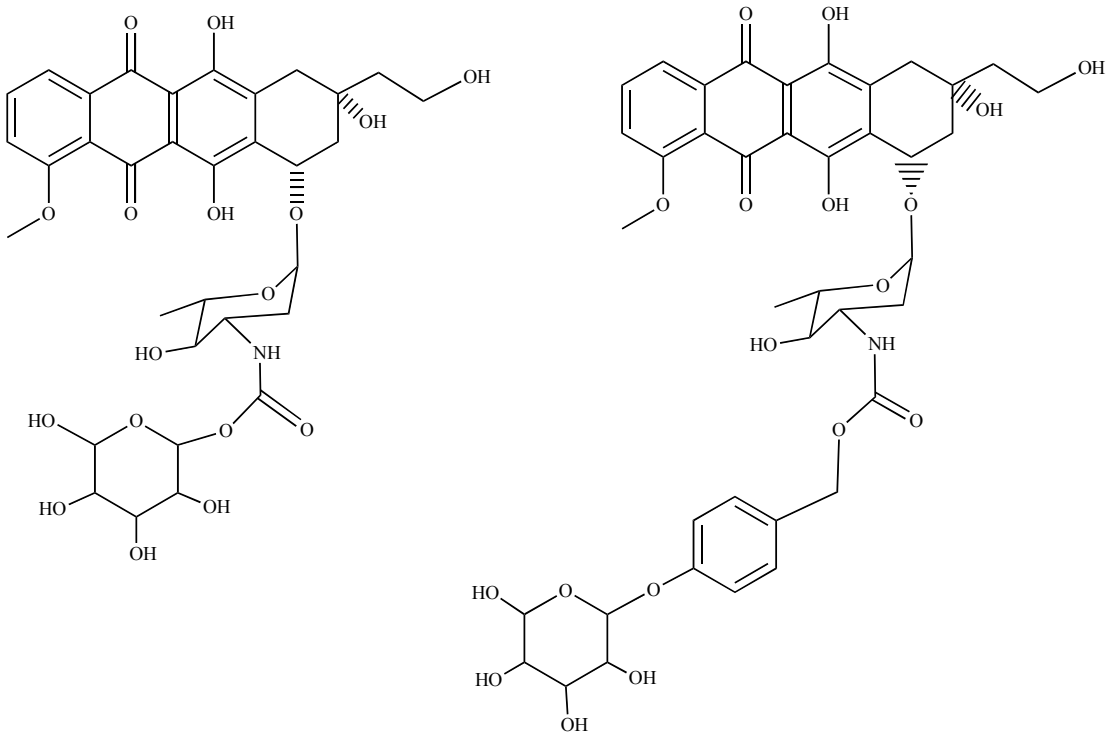


Fig. (7).

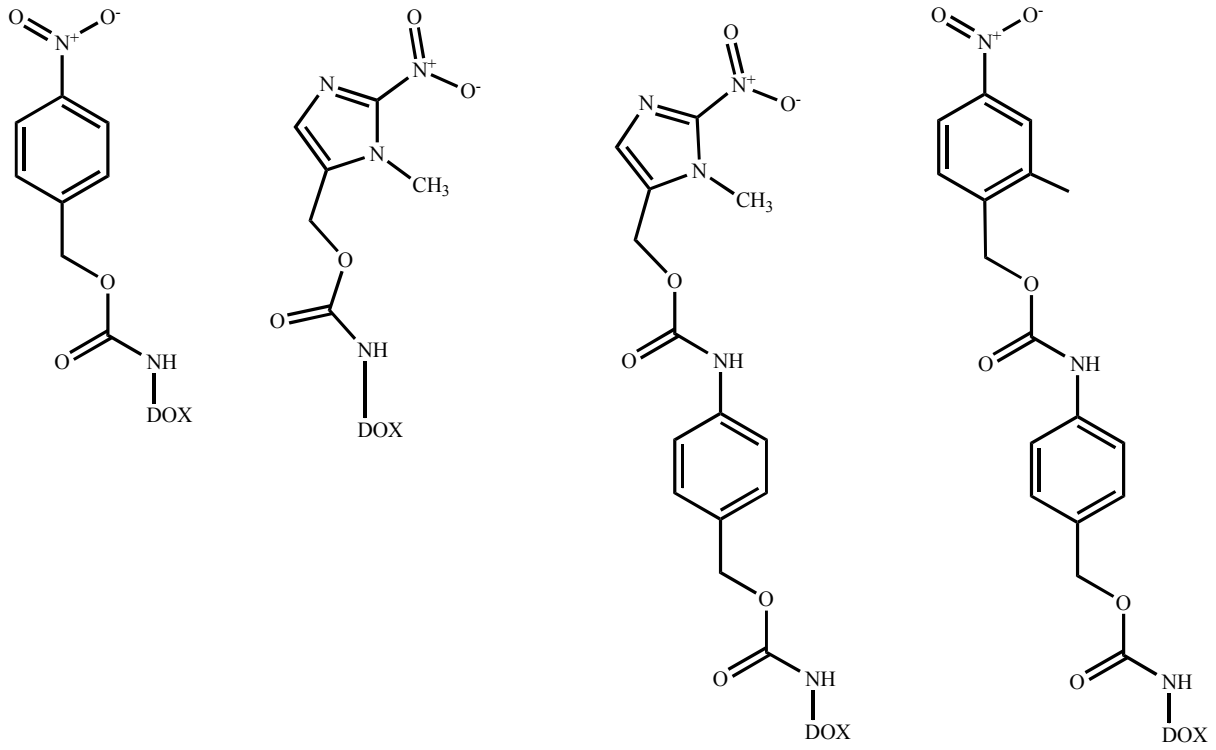


Fig. (8).

Garsky *et al*. [41] reported that DOX had a limited utility in prostate cancer due to systemic toxicities, primarily cardiotoxicity and myelosuppresion. The administration of a prodrug of DOX designed to permit selective activation by the tumor, would reduce general systemic exposure to the DOX. Serum prostate spesific antigen (PSA) levels have been found to correlate with the malignant prostate cells. In the mentioned study coupling of selected peptides to DOX provided series of DOX-peptide conjugates which were evaluated *in vitro* and *in vivo* as targeted prodrugs for PSA-secreting tumor cells. Glutaryl-Hyp-Ala-Ser-Chg-Gln-Ser-Leu-DOX (Fig. **10**), as the peptide-DOX conjugate was described as 20-fold selective against human prostate [41].

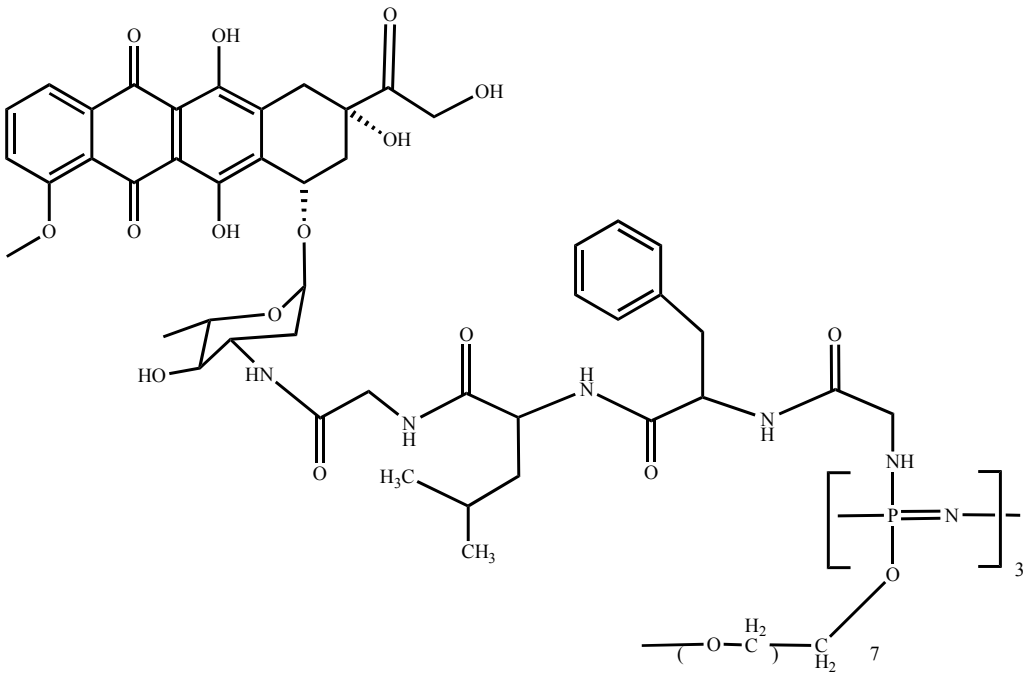


Fig. (9).

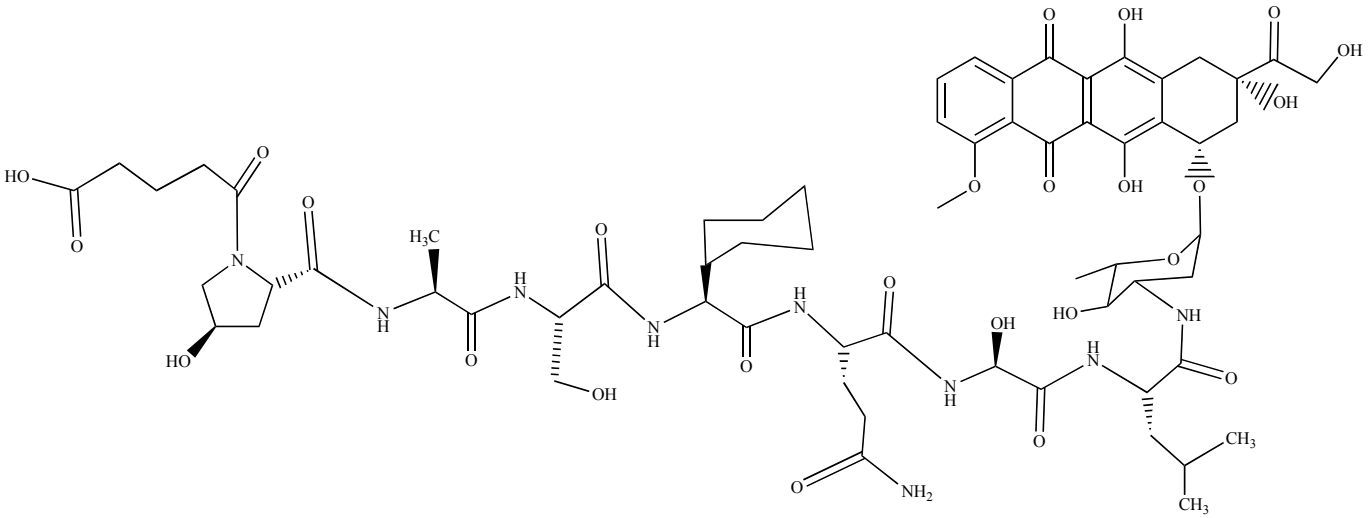


Fig. (10).

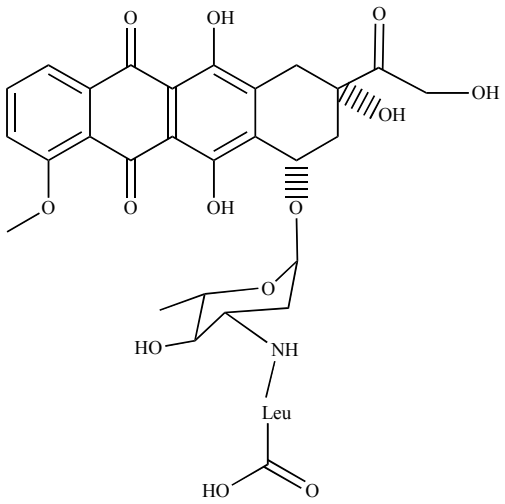
A peptide prodrug of DOX (Leu-Dox) transformed into DOX in tumor cells by cathepsin-like enzymes and exhibited superior antitumor activity than DOX. In addition, this prodrug revealed lower levels of cardiotoxic DOX in heart tissues. Tetrapeptide prodrug of DOX (Leu-Ala-Leu-Ala-Dox) was developed and its cleavage to free DOX by peptidases secreted from MCF7/6 human breast cancer cells was reported (Fig. **11**). Heptapeptide prodrug of DOX (L-377, 202) (Fig. **12**) was engineered to be a substrate for the serine protease PSA which is excreted from prostate cancer cells [42].

Etrych *et al*. [43] reported the synthesis and properties of new immunoglobulin-containing polymer-drug hydrazone conjugates of a star structure designed for antitumor therapy. DOX-HPMA copolymer conjugate (PK1) has a molecular weight of approximately 28 kDa and contains DOX linked through its amino sugar to the HPMA copolymer by a tetrapeptide spacer, Gly-Phe-Leu-Gly. This peptide sequence is cleaved by lysosomal enzymes of tumor cells. According to Haag and Kratz [44] PK2 is a related compound to PK1, a galactosamine group was designed to be taken up by the asialoglycoprotein receptor of liver tumor cells. Elastin-like polypeptide-DOX conjugates (ELP-DOX) have been designed by Furgeson *et al.* [45].

2.4. Other Prodrugs of Doxorubicin

The prodrugs of antitumor agents have been activated by oxidoreductases, transferases, hydrolases and lyases and nonhuman enzymes as nitroreductase [2]. Mauger *et al*. [46] reported the synthesis and properties of N-4-(nitrobenzyloxycarbonyl)doxorubicin for antibody-directed enzyme prodrug (Fig. **13**).

Doxorubicin-*N*-p-hydroxyphenoxyacetamide (DPO) and *N*-(phenylacetyl)DOX were synthesized as prodrugs of DOX



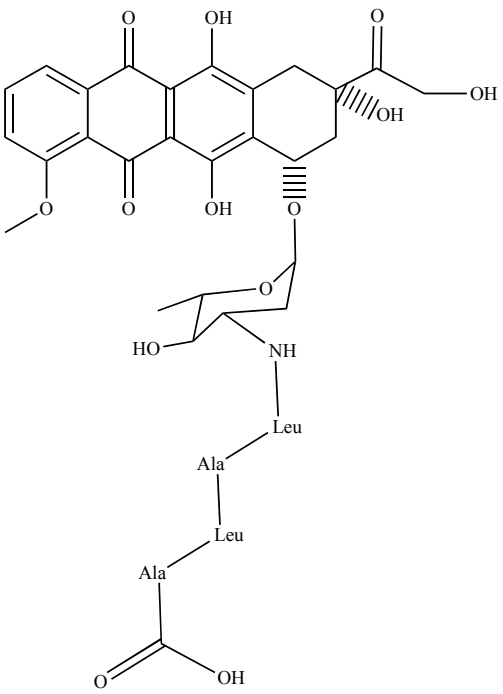


Fig. (11).

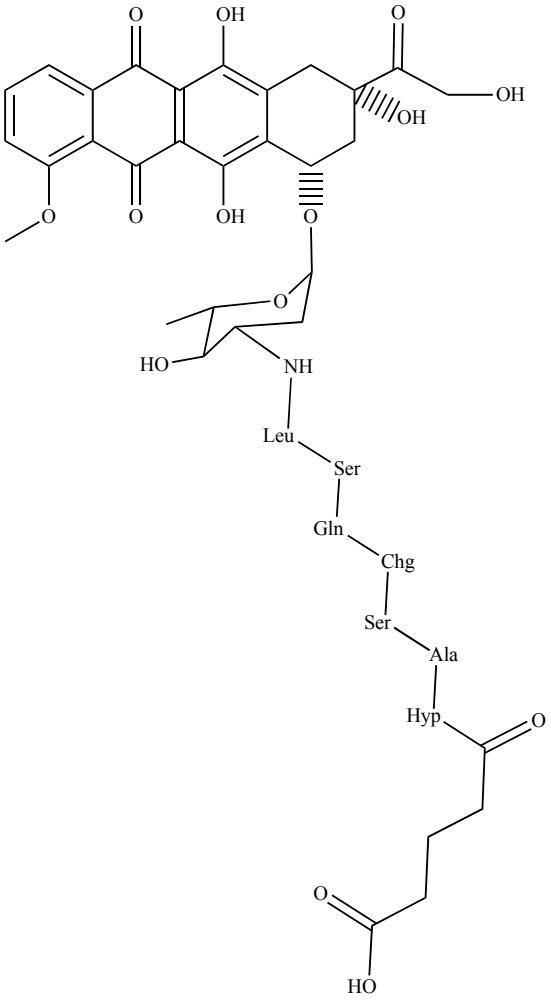


Fig. (12).

and shown to be activated by bacterial penicillin V amidase [47] and penicillin G amidase [48], respectively. DPO and *N*-(phenylacetyl)DOX were 20- to 80-fold and 10-fold less cyctoxic toward lung carcinoma and adenocarcinoma cells than DOX, respectively. More recently, DPO was used in another ADEPT approach by targeting penicillin V amidase to folate receptor-positive cells [49]. DPO was as toxic as DOX to folate receptor-positive cells whereas DPO was nontoxic towards folate receptor-negative cells.

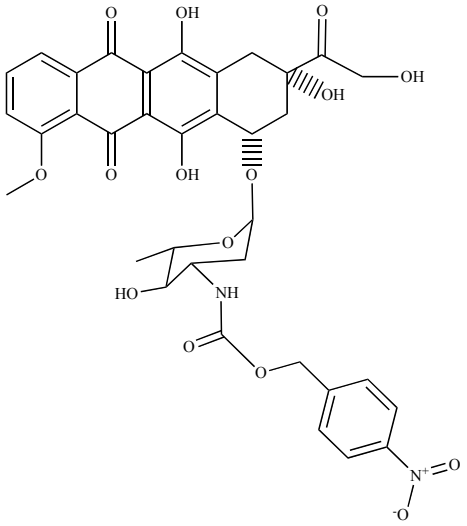


Fig. (13).

β-lactamase catalyzes the hydrolysis of β-lactams to substituted β-amino acids [50]. The enzyme, which is present in various bacteria, also hydrolyse cephalosporins and penicillins. β-lactamase is not present in human cells, and therefore only ADEPT approaches are suitable to target prodrugs that needs β-lactamase-mediated activation. The DOX prodrug was shown to be activated by β-lactamase and

was 20-fold less cytotoxic toward human breast cancer cells than DOX [51, 52].

The cephalosporin DOX prodrug C-DOX (BMY 46633) (Fig. **14**) was prepared and shown to be activated by β-lactamase from *E. cloacae* [53]. BMY 46633 was less toxic than the parent DOX in three different tumor cell lines.

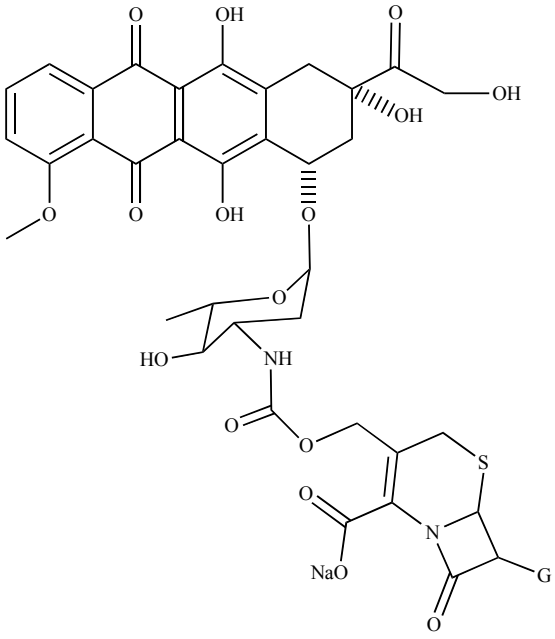


Fig. (14).

The most important review articles releated with doxorubicin prodrugs are about DOXO-EMCH (INNO-206) which is the first prodrug of doxorubicin to enter clinical trials [54] and anticancer carrier-linked prodrugs that phase I-III studies have been performed [35].

3. CONCLUSION

The number of publications on prodrugs have been increasing in the recent years. The prodrug approach has been observed in a lot of useful applications in drug research and development. In this paper, the releated articles and reviews of doxorubicin prodrugs have reported. Future researches may be focused on the pharmacokinetic studies of doxorubicin prodrugs.

ABBREVIATIONS

ADEPT	=	Antibody-directed enzyme prodrug therapy
DOX	=	Doxorubicin
DOX-GA3	=	*N*-[4-doxorubicin-*N*-carbonyl-(oxymethyl)-phenyl]-*O*-β-glucuronyl carbamate
DPO	=	Doxorubicin-*N*-p-hydroxyphenoxyacetamide
GDEPT	=	Gene-directed enzyme prodrug therapy
HMR 1826	=	*N*-[4-doxorubicin-*N*-carbonyl-(oxymethyl)-(4-nitrophenyl)]-*O*-β-glucuronyl carbamate
L-HSA	=	Lactosaminated human albumin
MC-DOXHZN, DOXO-EMCH	=	(6-maleimidocaproyl)hydrazone of DOX
PAMAM	=	Polyamidoamine
PSA	=	Prostate spesific antigen
VDEPT	=	Virus-directed enzyme prodrug therapy

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