Drugs of the Future 2004, 29(4): 343-357

CCC: 0377-8282/2004

Review Article

Application of organic carbamates in drug design. Part 1: anticancer agents – recent reports

Suprabhat Ray[1] and Devdutt Chaturvedi[2]

Medicinal Chemistry Division , Central Drug Research Institute, Lucknow 226 001, India.

CONTENTS

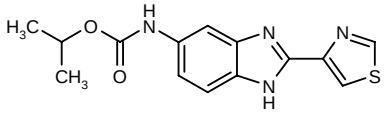
Abstract

Organic carbamates can be used as anticancer, antimicrobial and antimalarial agents, as well as in CNS/CVS disorders and many other areas, either in the form of drugs *per se* or as prodrugs. As anticancer agents, carbamate chemistry has mainly been used to develop prodrugs. Different mechanism-based approaches have been developed for preparing cytotoxic site-directed drugs of natural as well as synthetic origin. In the preparation of prodrugs, the free phenolic group, generally responsible for causing cytotoxicity in anticancer drugs, is masked in the form of a carbamate ester. The molecule is designed so that the release of the active drug takes place only at the tumor site. Thus, normal cells escape exposure to toxicity causing side effects. Various approaches have been adopted wherein the molecular transformation caused by biochemicals/enzymes at the tumor site releases the active drug. The present review is limited to the use of carbamates as anticancer agents and covers only reports from 1990 onwards. Both natural and synthetic products having a carbamate residue and their potential anticancer activity are discussed, as well as the chemistry behind the mechanism of release of the active component from the prodrugs.

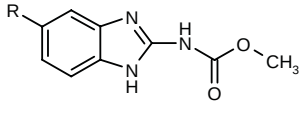
Introduction

The esters of carbamic acid with substitutions at the amino and the ester ends (NHRCOOR') form a class of compounds referred to as organic carbamates. Many carbamates have found use in different areas such as agrochemicals (1), intermediates in organic synthesis (2), protecting groups, particularly in peptide synthesis (3), linkers in combinatorial chemistry (4) and as pharmaceuticals (5-7). In the field of pharmaceuticals, the introduction of the benzimidazole carbamates cambendazole **(1)**, mebendazole **(2)**, flubendazole **(3)**, albendazole **(4)**, *etc.*, as anthelmintics (8) in the 1970s is an important milestone.

In recent years, several reports have appeared on carbamates in relation to drugs indicating their growing importance in drug research. The areas in drug research where carbamates have found extensive application include: cancer, bacterial and viral infections and central nervous system/cardiovascular disorders. This review is not exhaustive, but covers only recent important reports from 1990 onwards on the use of carbamates as anticancer drugs.



(1)



(2) R = COPh
(3) R = 4-F-PhCO
(4) R = SPr

[1]*Present address: 16/3 kh, Sarojini Naidu Marg, Masonic Lodge Compound, Lucknow 226 001, India; e-mail: suprabhatray@yahoo.co.in;* [2]*Present address: 374, College of Pharmacy, University of Georgia, Athens, Georgia 30602-2352, USA; e-mail: ddchaturvedi002@yahoo.co.in.*

Carbamates as anticancer agents

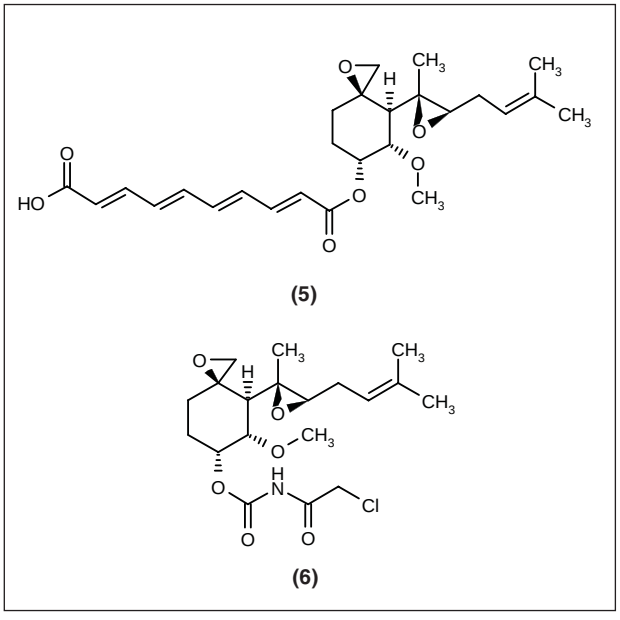
One approach to cancer chemotherapy is the use of cytotoxic agents. Although cancer cells are preferentially killed by cytotoxic compounds, they are associated with damage to normal cells as well. It is therefore necessary that: 1) the cytotoxic compound be released only at the desired site; 2) the cytotoxic effect be reasonably potent so that cancer cells are eliminated; and 3) the half-life of the drug not be too long to allow it to disperse to other sites, resulting in systemic toxicity. Keeping the above criteria in mind, carbamate compounds have been mainly developed as prodrugs, although some may be active themselves. The anticancer carbamates are therefore covered under the following two categories: carbamate derivatives as anticancer agents *per se* and carbamate prodrugs as anticancer agents.

Carbamate derivatives as anticancer agents per se

1. Anticancer carbamates related to natural products

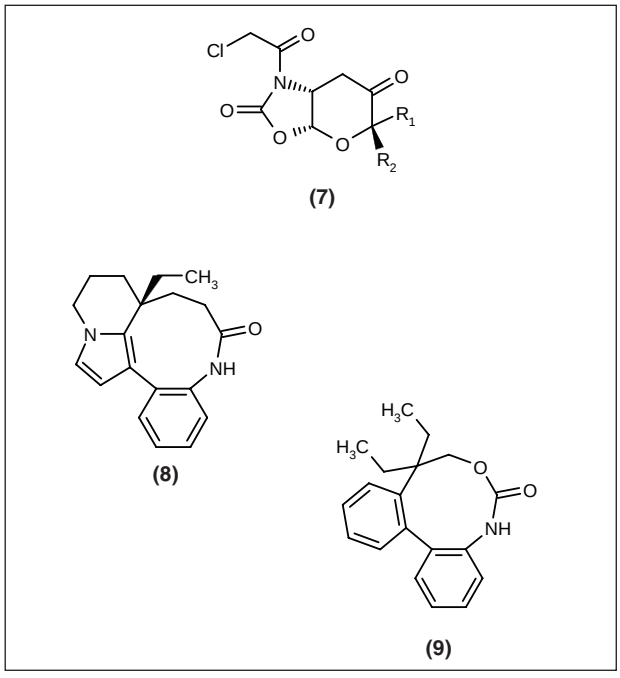
a. Fumagillin analogues

The natural antibiotic fumagillin **(5)** inhibits endothelial cell proliferation *in vitro* and tumor-induced angiogenesis *in vivo*, as well as tumor growth in mice. Prolonged administration causes weight loss, necessitating structural modification. Replacement of the unsaturated ester chain by an *O*-(chloroacetyl)carbamoyl moiety resulted in the potent anticancer compound TNP-470 **(6)**, which is 50 times more active than fumagillin and devoid of its side effects (9). TNP-470 was studied in clinical trials (10). The antiangiogenic activity of TNP-470 was retained when the methoxy residue was replaced by a carbohydrate pyranyl ring (11). Cyclic analogues **(7)** of TNP-470 were recently prepared (12). However, most of the compounds were less active than fumagillin, except R_1 = C≡C-C_5H_{11}, R_2 = CH_3, which was equiactive against the human tumor cell line A-431.



(5)

(6)



(7)

(8)

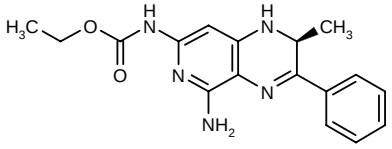
(9)

b. Ring biaryl carbamate analogues of rhazinilam

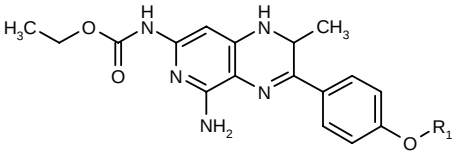
The antitubulin property of the alkaloid (–)-rhazinilam **(8)** is responsible for its marked cytotoxic activity towards cancer cells. However, it showed no *in vivo* activity. To improve upon its activity, cyclic biaryl carbamate analogues of rhazinilam were synthesized (13). The most active compound resulting from this approach was compound **9,** which exhibited 2-fold greater activity on microtubule disassembly compared to **8**, and similar cytotoxicity.

2. Anticancer carbamates of synthetic origin

The pyrido[3,4-*b*]pyrazine carbamate **10** has entered phase I clinical trials as a potent antimitotic agent (14). The metabolism of **10** in mice produced the hydroxyphenyl derivative as a major active metabolite (15). This led to the preparation of various *O*-substituted phenyl derivatives (**11**, [*RS*]- and [*S*]-isomers), resulting in several potent anticancer compounds. The benzyl ether **11c** showed decreased cytotoxicity (> 14-fold) and enhanced antimitotic activity (> 15-fold) in cultured cells and a greater increase in life span (~ 2-fold) in mice as compared to **11a,** (*RS*)**-11b** and (*S*)**-11b**. Bulky substitution in the carbamate moiety (16), substitution on the 5-amino group (17) or substitution in place of 3-phenyl (18) caused a reduction in biological activity.



(10)

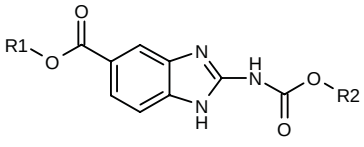


(11)
a: R_1 = H
b: R_1 = Me
c: R_1 = Bz

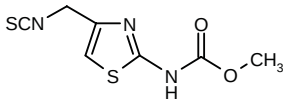
a. Alkyl-5-(alkoxycarbonyl)-1*H*-benzimidazole-2-carbamates

Inhibition of microtubule assembly, the mechanism for anthelmintic activity, is due to mitotic arrest, resulting in inhibition of cell growth. Therefore, certain alkyl-5-(alkoxycarbonyl)-1*H*-benzimidazole-2-carbamates **(12)**, which were basically developed as antifilarials, were tested for cell growth inhibition and found active against the murine leukemia L1210 cell line, with IC_{50} values below 1 μM (19). Growth inhibition by this series of compounds appeared to be associated with mitotic spindle poisoning.

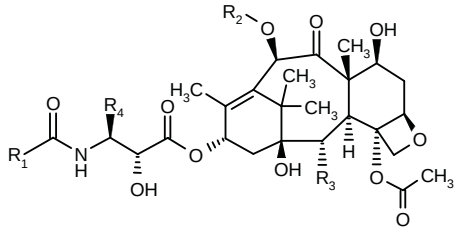
Similarly, methyl 4-(isothiocyanatomethyl)thiazole-2-carbamates **(13)**, also prepared as antifilarial agents, inhibited the growth of leukemia L1210 cells with an IC_{50} value of 3.2 μM (20).



(12)
a: R_1 = i-Pr, R_2 = Me
b: R_1 = $(CH_2)_2CH(CH_3)CH_2C(CH_3)_3$, R_2 = Me
c: R_1 = Et, R_2 = Et
d: R_1 = i-Pr, R_2 = i-Pr



(13)



(14)
a: R_1 = Ph, R_2 = Ac, R_3 = OBz, R_4 = Ph
b: R_1 = t-BuO, R_2 = H, R_3 = OBz, R_4 = Ph
c: R_1 = t-BuO, R_2 = H, R_3 = OBz, R_4 = t-Bu
d: R_1 = $OCEt(Me)_2$, R_2 = H, R_3 = OBz, R_4 = t-Bu
e: R_1 = t-BuO, R_2 = Ac, R_3 = 3-MeO-Ph-CONH, R_4 = Ph
f: R_1 = t-BuO, R_2 = Ac, R_3 = 3-Cl-Ph-CONH, R_4 = Ph

Carbamates as prodrugs in anticancer therapy

1. Carbamates of natural anticancer agents as prodrugs

a. Prodrugs of paclitaxel and docetaxel

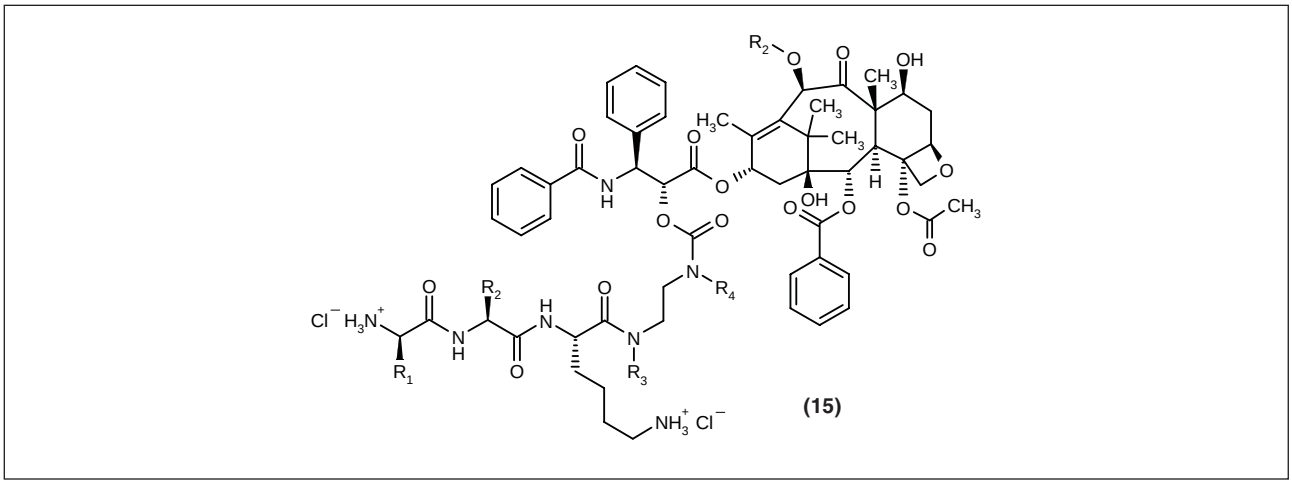
The diterpenoid paclitaxel **(14a)** (21) and its semisynthetic analogue docetaxel **(14b)** (22) are used clinically for the treatment of breast, ovarian and lung cancers. However, like other cytotoxic agents, paclitaxel causes serious side effects and is poorly soluble in water. The carbamate docetaxel is twice as active (25, 26). From a series of other carbamate derivatives (27), the most potent compounds were **14c** and **14d,** which were as active as docetaxel but 4-5 times more soluble in water.

In a different approach, prodrugs of docetaxel have been prepared through introduction of 2-amido groups (26) in place of benzyloxy. Among these, 3-methoxy and 3-chlorobenzoylamido analogues **14e** and **14f**, respectively, were the most active, but not superior to docetaxel and paclitaxel.

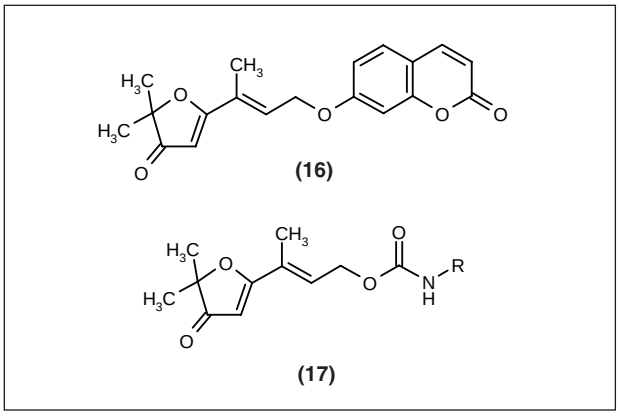
In a further modification, highly potent cytotoxic taxoids were obtained by replacing 3'-phenyl by a trifluoromethyl group in docetaxel, and different substitutions for R_2 (27). The enhancement of activity was significant against the multidrug-resistant (MDR) breast cancer cell line MCF7R expressing the MDR phenotype. Similar effects were observed upon variation of substituents at C-10 in 3'-alkyl and 3'-alkenyl series (28). In a targeted approach, paclitaxel derivatives **15** were prepared with a 2'-carbamate chain having a residue that is hydrolyzed only in the presence of the tumor-specific enzyme plasmin and not under the influence of other enzymes distributed ubiquitously throughout the body (29).

b. Geiparvarin analogues

Geiparvarin **(16),** isolated from the plant *Geijera parviflora* (30), shows antitumor properties. In a study of structural analogues, the coumarin ring was replaced by



(15)



(16)

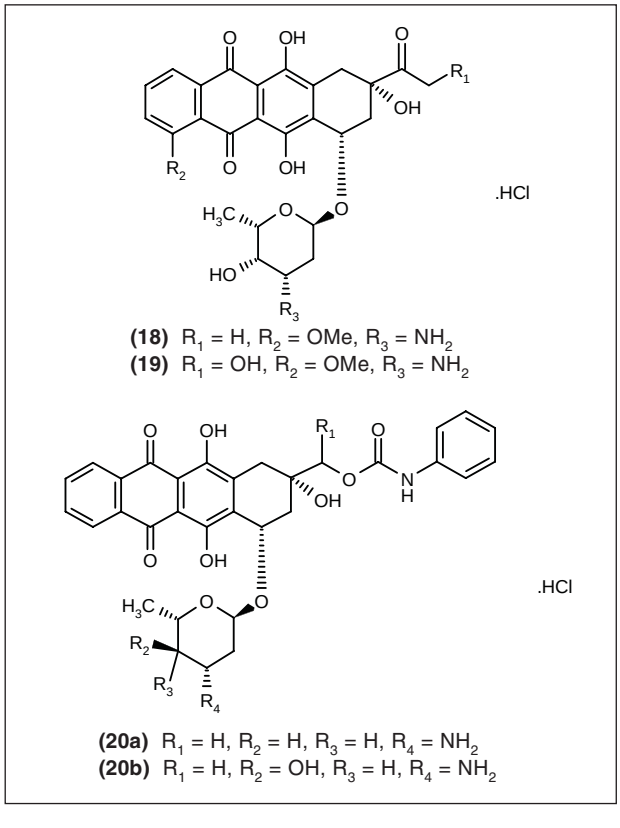
(17)

a carbamate moiety, a bioisostere of an allylic fragment. The *N*-alkylcarbamates **17** were endowed with potent cytostatic activity *in vitro* against murine (L1210, FMSA) cell lines (31), whereas *N*-arylcarbamates were inactive.

c. Anthracycline derivatives

The anthracyclines daunorubicin (daunomycin, **18**) and doxorubicin (adriamycin, **19**) are employed clinically in the treatment of cancer (32). However, these antibiotics cause undesirable side effects, particularly cardiotoxicity. In attempts to reduce their side effects, various structural modifications have been carried out. Since their major active metabolites involve reduction of the C-13 oxo group, derivatives of 9-hydroxyalkyl analogues were synthesized (33). *N*-Phenylcarbamates of 9-hydroxyalkyl analogues of daunorubicin and doxorubicin showed promising activity against L1210 leukemia in mice. A high order of activity was observed in phenylcarbamates having a modified sugar residue attached. Thus, **20a** and **20b** showed a T/C of 500% and 560%, respectively, at doses of 2 and 1 mg/kg i.p., values which were significantly higher than those for the parent compounds **18** and **19**. Preliminary studies showed that **20b** possessed broad-spectrum antitumor activity when administered orally and was less cardiotoxic compared to the parent compound.

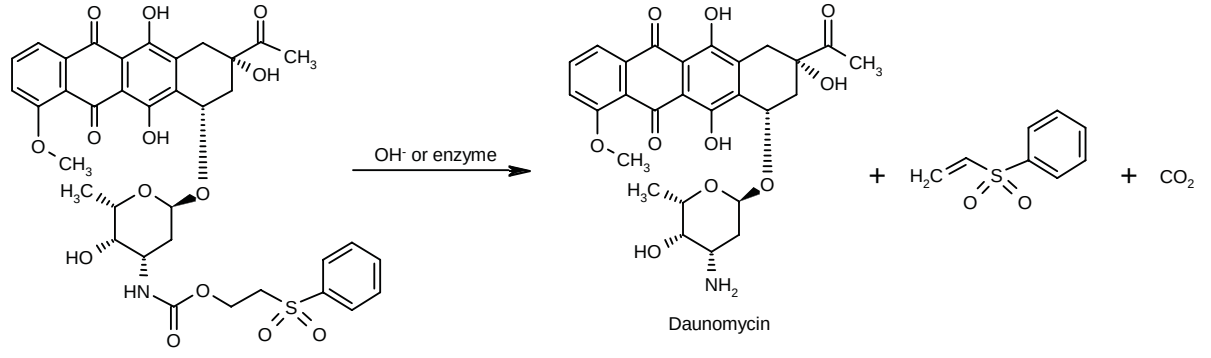
In a tumor-specific approach, the amino group of daunorubicin was blocked by a phenylsulfonylethoxycarbonyl group. Such groups have been found to be a useful triggering device for activation of the compound within the cell (Scheme 1). Daunorubicin phenylsulfonylethoxycarbamate **(21)** was found to have biological activity comparable to that of the parent compound, while showing considerably lower systemic toxicity in mice (34).



(18) R_1 = H, R_2 = OMe, R_3 = NH_2
(19) R_1 = OH, R_2 = OMe, R_3 = NH_2

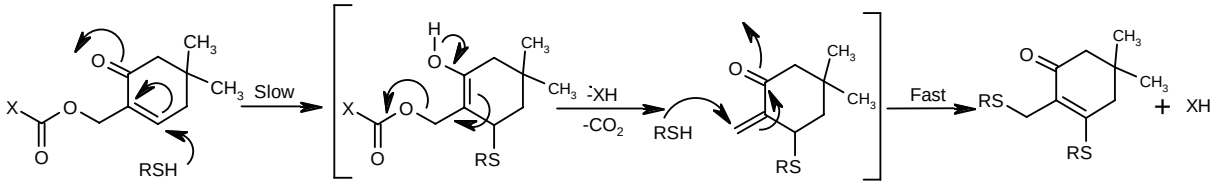
(20a) R_1 = H, R_2 = H, R_3 = H, R_4 = NH_2
(20b) R_1 = H, R_2 = OH, R_3 = H, R_4 = NH_2

Scheme 1



(21)

Scheme 2



(22)

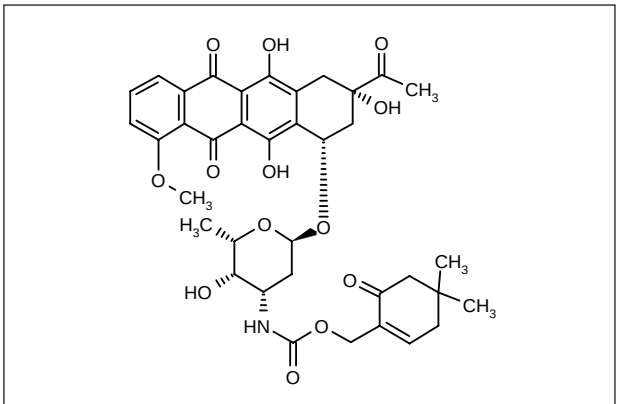
XH = antineoplasic agent RSH = sulfhydryl compound such as GSH

A mechanism for development of resistance to antineoplastic agents is their increased detoxification caused by intracellular glutathione (GSH), sulfhydryl compounds such as GSH and metallothionein (MT). Such compounds are known to react with α,β-unsaturated ketones and esters. An increase in GSH in tumor cell lines with acquired resistance to doxorubicin has been observed (35). It was therefore postulated that intracellular GSH may have a carbon-acyloxy bond in **22,** releasing the drug at the site (Scheme 2).

Based on the above assumption, the carbamate prodrug **23** was synthesized (36) and found to be less toxic than the free drug against L1210 cells. A doxorubicin-resistant L1210 cell line was more sensitive to **23** than to free daunorubicin, with a relative resistance (IC_{50} resistant cell line/IC_{50} parental cell line) of 3.7 and 9.2, respectively.

In yet another approach, glucuronylated daunorubicin was prepared (37) as a prodrug for antibody-directed enzyme prodrug therapy (ADEPT). In this technique, reported by Connors (38) and re-emphasized in the case of antitumor drugs by Bagshawe *et al.* (39) and Senter *et al.* (40), a monoclonal antibody (MAb) directed against a particular tumor and covalently bound to a prodrug-clearing enzyme is first injected to localize at the tumor cell surface antigen. Subsequently, a prodrug with marked toxicity is administered and the cytotoxic species cleaved by the enzyme is released on the tumor cell surface.

Thus, a fusion protein prepared from the anti-CEA MAb BW431 and β-glucuronidase as antibody-directed

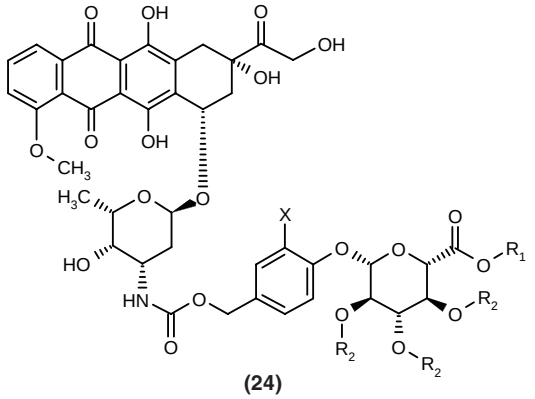


(23)

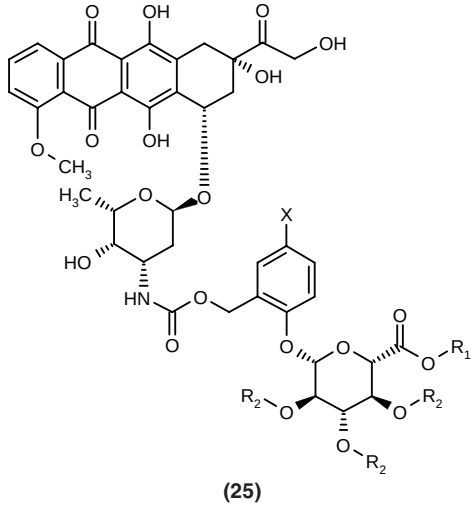
enzyme was first administered followed by doxorubicin prodrugs **24** and **25**. The most active compound was **24** (R_1=R_2 = H, X = NO_2; HMR-1826), which is in clinical trials. It inhibited the growth of L1210 leukemia cells *in vitro* with an IC_{50} of 2.21 μM compared to a value of 0.03 μM for doxorubicin, but with 100-fold reduced toxicity.

d. Etoposide prodrugs

Etoposide (**26**) is a semisynthetic derivative of podophyllotoxin with established indications of testicular



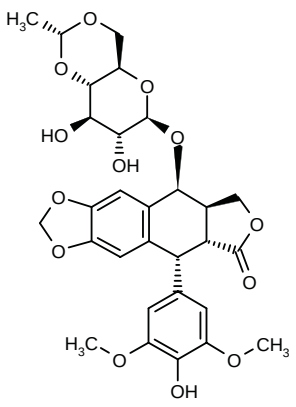
(24)



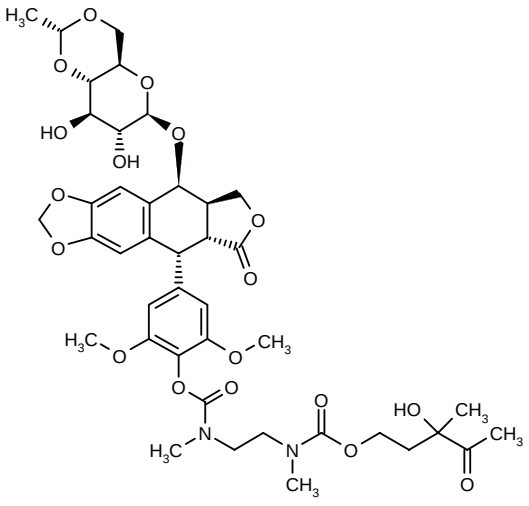
(25)

and small cell lung cancer, in pediatrics for the treatment of neuroblastoma, and also leukemia and Kaposi's sarcoma. Its poor water solubility is a drawback in its clinical use.

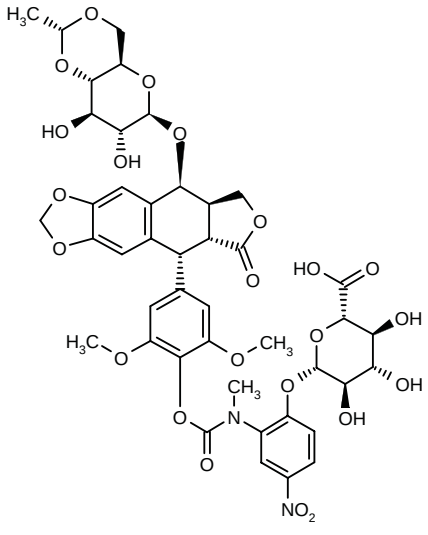
To reduce the toxic effects of etoposide, its prodrug **27** was prepared. The prodrug incorporates a trigger portion designed to be released by a sequential retro-aldol/retro-Michael reaction catalyzed by the aldolase antibody 38C2. The prodrug is 100-fold less toxic than etoposide *in vitro* against the NXS2 neuroblastoma cell line. The activity of etoposide was restored after activation by antibody 38C2. When the prodrug was administered to mice with established tumors after injecting the antibody, a 75% reduction in tumor growth was obtained (41). In another tumor-specific design based on prodrug monotherapy, which utilizes specific enzymes present in cancer cells, such as β-glucuronidase, in high local concentration, the etoposide prodrug **28** linked to β-glucuronide through a "self-immolative" spacer was developed. *In vitro*, the prodrug was shown to be less cytotoxic and more water-soluble than etoposide. In the presence of the β-D-glucuronidase, cleavage of the prodrug with complete release of the parent drug was observed (42).



(26)



(27)

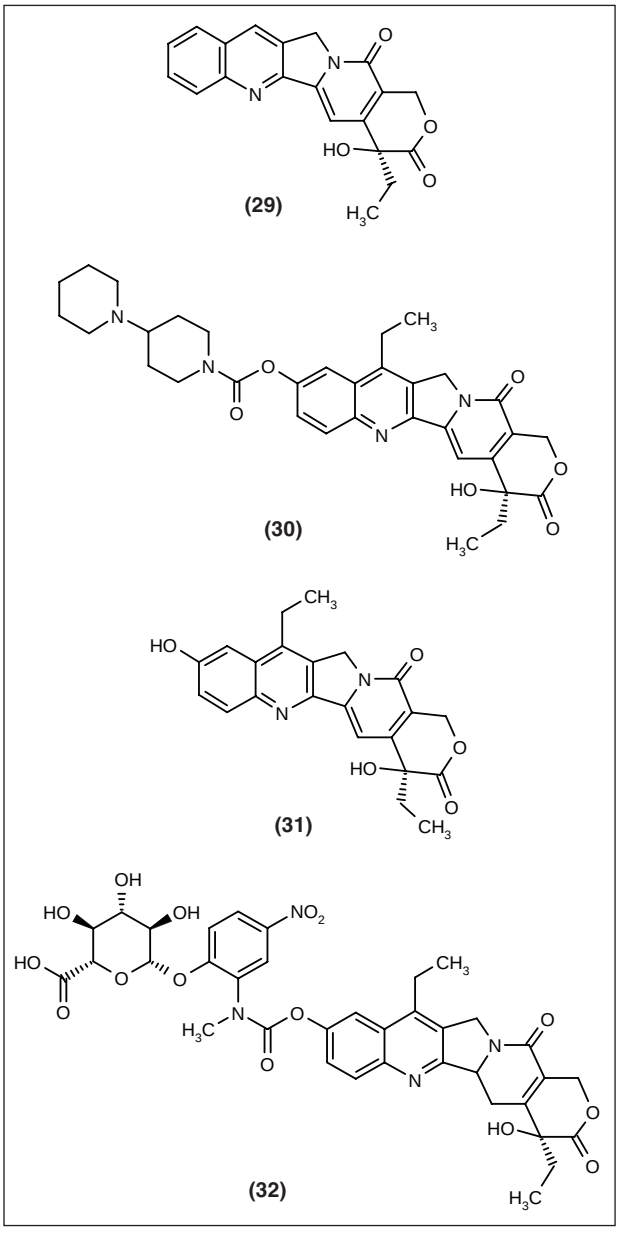


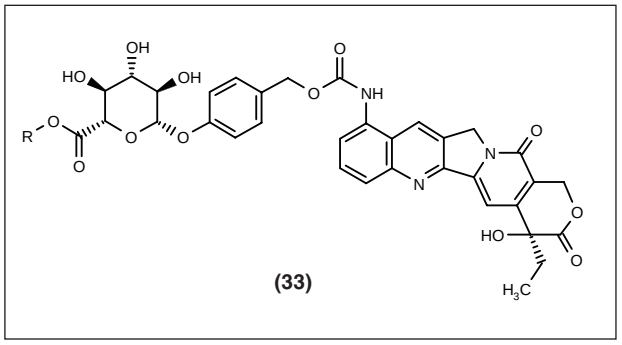
(28)

e. Camptothecin prodrugs

The alkaloid camptothecin **(29)** (43) inhibits topoisomerase I and displays antitumor activity. It could not be developed into a drug due to its non-mechanism-related toxicity and poor water solubility. To circumvent these problems, many derivatives have been prepared. The carbamate derivative irinotecan (CPT-11, Campto®, **30**) (44) has found use in several indications, including colon and ovarian cancer. It has also been shown to reduce angiogenesis (45).

Since irinotecan is a prodrug of 10-hydroxycamptothecin **(31)**, a glucuronide derivative of **31** with a spacer in between was prepared (46). Prodrug **32** was found to be 70-fold less toxic compared to **31**, but in the presence of enzyme its cytotoxicity was comparable.





A prodrug of 9-aminocamptothecin (**33**) was prepared (47) as a glucuronide derivative linked through a "self-immolative" spacer to release the drug at the tumor site. Prodrug **33** (R = H) and its potassium salt **33** (R = K) were 20-80-fold less toxic than 9-aminocamptothecin to human tumor cell lines. The simultaneous addition of β-glucuronidase and **33** to tumor cells, however, resulted in a cytotoxic effect equal to that of the parent compound. The prodrugs were 80 (R = H) to 4,000 (R = K) times more soluble in water at pH 4.0.

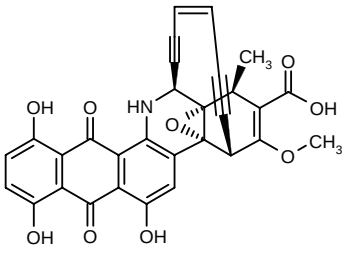
f. Dynemicin analogues: enediyne compounds

The cyclic enediyne antibiotics are fast emerging as a novel class of anticancer compounds. The simplest member, dynemicin A **(34)**, shows high potency against various tumor cell lines and significantly prolongs the life span of mice inoculated with P388 leukemia and B16 melanoma cells. In these compounds, cycloaromatization of the enediyne moiety leads to the formation of a benzenoid diradical, which removes hydrogen from the DNA strand to initiate its rupture.

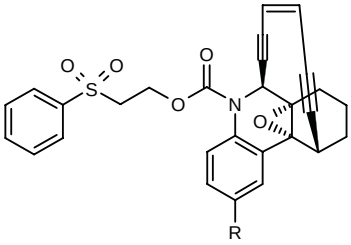
Recent work on dynemicin A and its analogues has shown that this Bergman cycloaromatization step can be controlled by a suitably designed unit, such as carbamate, acting as a trigger that releases the reactive enediyne under the influence of biochemicals, enzymes or photolytic conditions, producing cytotoxicity. It was hypothesized that different cells might possess varying degrees of activating power towards initiation and that DNA damage might occur in a cell-specific manner.

A number of potent carbamate esters of enediynes have been reported (48a-d). Compounds **35** and **36** (48a) and their cytotoxicities against Molt-4 leukemia cells are shown below. The sulfone derivatives undergo biochemically induced β-elimination of phenyl vinyl sulfone, generating the cytotoxic drug **37**.

The release of the active drug has also been effected through an antibody- or gene-directed enzyme prodrug (49a, 49b). A tumor-specific antibody linked to the enzyme, an aerobic nitroreductase from *Escherichia coli* B, is first administered together with NADH or NADPH, followed by the prodrug **38**, with the quinoline nitrogen protected by a 4-nitrobenzyloxycarbonyl group. The

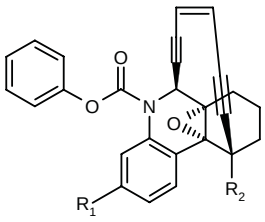


(34)

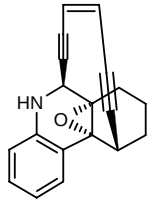


(35)

R = OMe IC_{50} = 6.5 nM
R = OCH_2CH_2OH IC_{50} = 0.02 pM



(36)



(37)

R_1 = OH, R_2 = OAc IC_{50} = 60 nM
R_1 = t-$BuCO_2$,R_2 =H, IC_{50} = 35 pM

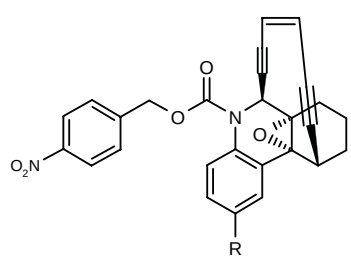
hydroxylamine, generated from nitro on reduction, fragments to give the active drug **39**.

Cleavage of the *N*-protective group in the dynemicin analogue **40** has also been reported through photolysis under neutral conditions (Scheme 3) (50, 51).

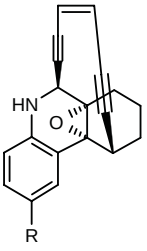
2. Carbamates of synthetic anticancer agents as prodrugs

a. Nitrogen mustards

Nitrogen mustards form a family of drugs useful in cancer therapy. The cytotoxicity of nitrogen mustards is



(38)

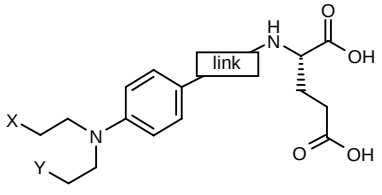


(39)

dose-related, they are associated with less resistance than other classes of anticancer agents, and they are highly effective against quiescent cancer cells. Various attempts have been made to reduce their toxicity by developing prodrugs. Some of the carbamate prodrugs are discussed below.

One of the nitrogen mustard prodrugs in clinical trials is compound **42a**. To improve further upon its activity, the corresponding carbamate **42b** was developed. The drug generated from **42b** under the influence of the enzyme carboxypeptidase G2 (CPG2), which is fused to the tumor-specific antibody, is 100-fold more active than the drug obtained from **42a** (52).

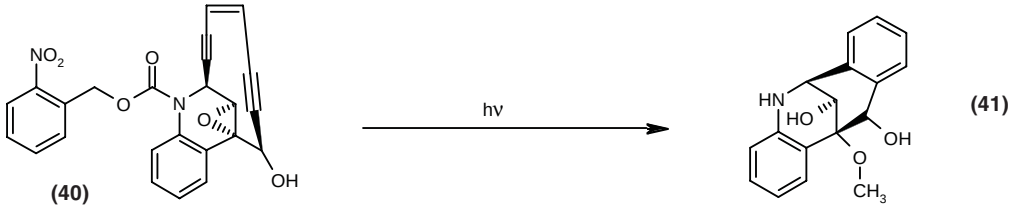
In another antibody-directed enzyme prodrug therapy, the phenolic mustard was prepared by linking it to β-glucuronide through a spacer to give compound **43** (53). Under the influence of antibody-linked β-glucuronidase, phenol is released, which then effects the release of the active phenolic mustard **44**. Prodrug **43** showed reduced toxicity against LoVo colon cancer cells (IC_{50} > 830 μM). After cleavage with β-glucuronidase, however, its



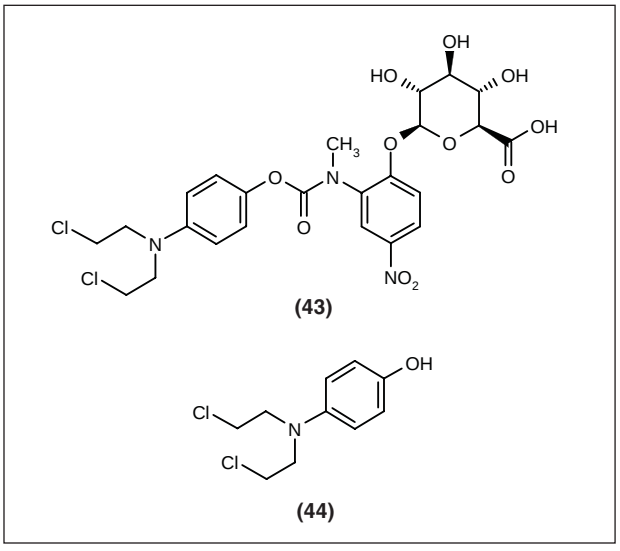
(42)

a: X = Cl, Y = $MeSO_3$, link = CO
b: X = Cl, Y = Cl, link = OCO

Scheme 3



(40) (41)



(43)

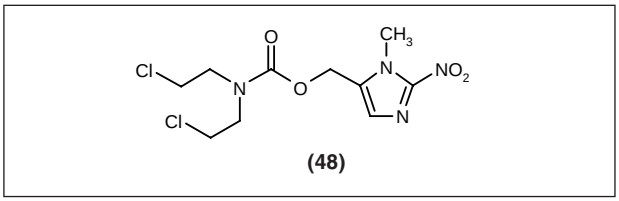
(44)

cytotoxicity was the same as that of the free drug (IC_{50} = 10.5 μM) (53).

In another prodrug, **45**, a different spacer was used. However, this prodrug failed to produce the desired cytotoxicity upon treatment with the enzyme. This was because the intermediate carbamic acid formed did not decarboxylate to produce the nor-nitrogen mustard **46**, but instead it cyclized to form the oxazolone **47** (Scheme 4) (54).

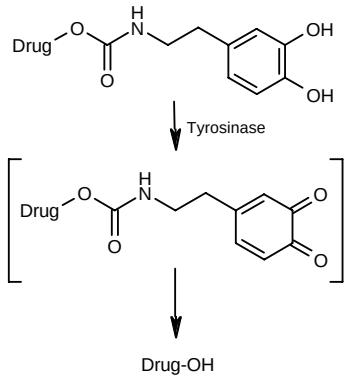
A hydroxymethylimidazole unit has been used as a trigger in **48**, the prodrug of *N,N*-bis(2-chloroethyl)amine (55). Bioreduction of the nitro group under hypoxic conditions and subsequent fragmentation produces the nitrogen mustard **46** (see later section).

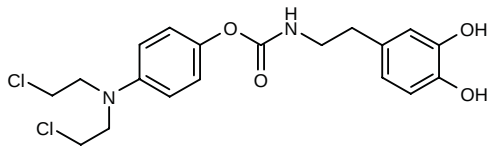
Yet another targeted delivery of prodrug utilizes the enzyme tyrosinase present in malignant melanoma cells for the release of the active drug to the site. A catechol or phenolic moiety, linked through a carbamate to the drug, would undergo oxidation by tyrosinase to release the active drug (Scheme 5). Thus, nitrogen mustard prodrug **49** was prepared (56) and evaluated for its biological activity. It showed increased cytotoxicity against tyrosinase-upregulated lines, compared with cell lines displaying little or no tyrosinase activity.



(48)

Scheme 5





(49)

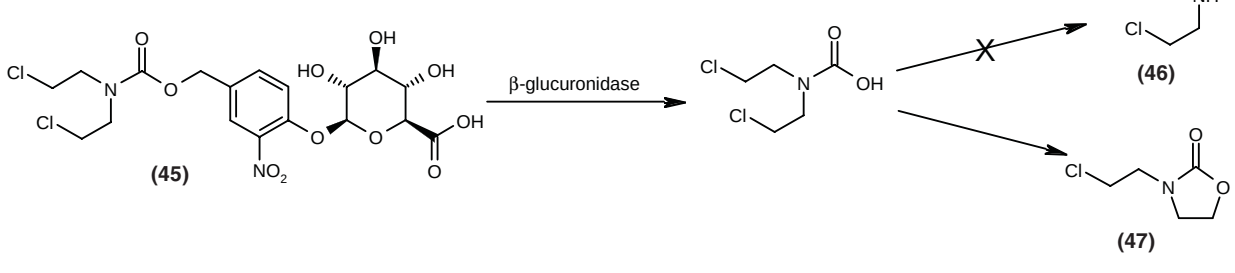
b. *N*-(2-Chloroethyl)-*N*-nitrosocarbamates

Certain new (2-chloroethyl)nitrosocarbamates having monomustard chains were prepared as prodrugs with improved water solubility and activity. The carbamic acid esters **50a** and **51** showed activity against tumor cell lines *in vitro* but were inactive in an *in vivo* assay against M5076 sarcoma in mice (57).

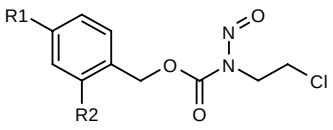
c. Pyrrolo[2,1-*c*][1,4]benzodiazepine prodrugs

High antitumor activity was encountered in the pyrrolo[2,1-*c*][1,4]benzodiazepine group of antibiotics

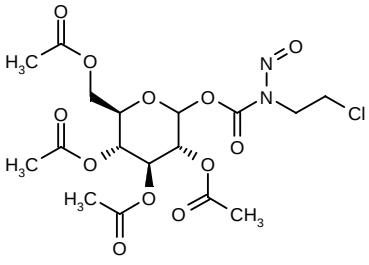
Scheme 4



(45) (46) (47)



(50)
a: R_1 = OAc, R_2 = H
b: R_1 = H, R_2 = NO_2



(51)

including DC-81 **(52)**, tomaymycin **(53)** and the dimer analogues DSB-120 **(54a)** and SJG-136 **(54b)**. *N*-Protected prodrugs of these antibiotics have been prepared (58) as the 4-nitrobenzylcarbamates **55, 56** and **57**, respectively. Such prodrugs are activated under the influence of the enzyme nitroreductase in the presence of the cofactor NADH or NADPH.

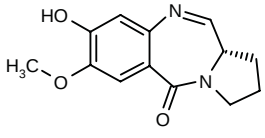
The prodrugs were found to be nontoxic in the human adenocarcinoma cell line LS 174T, whereas in the presence of the enzyme and the cofactor they were ~100-fold more toxic, although not to the full extent of their active counterparts **52-54**.

d. DNA-directed biscarbamate esters of pyrrolizines and imidazoles

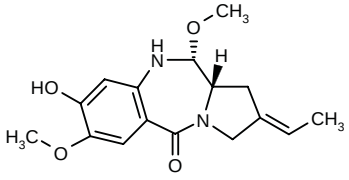
Bis(hydroxymethyl)pyrrolizine **58** and its biscarbamate **59** are potent alkylating agents. Another alkylating agent under clinical evaluation is thioimidazole (carmethizole, **60**) (59). To better target their interaction with DNA, they have been linked to either the DNA-intercalating carrier 9-anilinoacridine (compounds **61** and **62**) or the DNA minor groove-binding carrier 4-(4-quinolinylamino)benzamide (compounds **63** and **64**) (60). The former compounds were, in most cases, more potent. The cytotoxicities correlated broadly with the reactivities of the alkylating units.

e. Prodrug of amino-*seco*-CB1-TMI

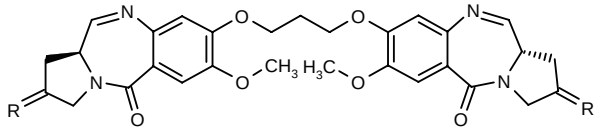
Amino-*seco*-CB1-TMI (**65**) is a potent minor groove-binding alkylating agent (61). In an effort to reduce its toxic effects and render it tumor-specific, the amino function was masked with a 2-nitroimidazole unit to form prodrug **66** (62). This 2-nitroimidazole residue serves as a substrate for the enzyme nitroreductase in the presence of NADH or NADPH, to generate the cytotoxic agent. The initial step, *i.e.,* conversion of NO_2 to NHOH, is inhibited by oxygen, thus providing the basis for hypoxic selectivity (Scheme 6) (63).



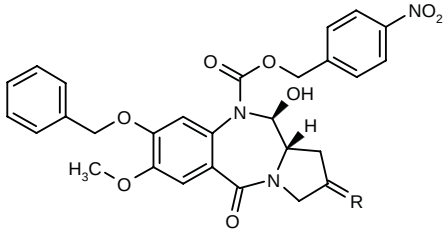
(52)



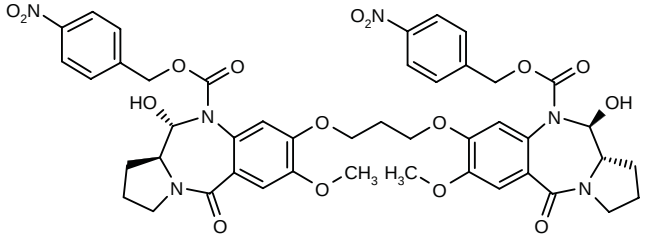
(53)



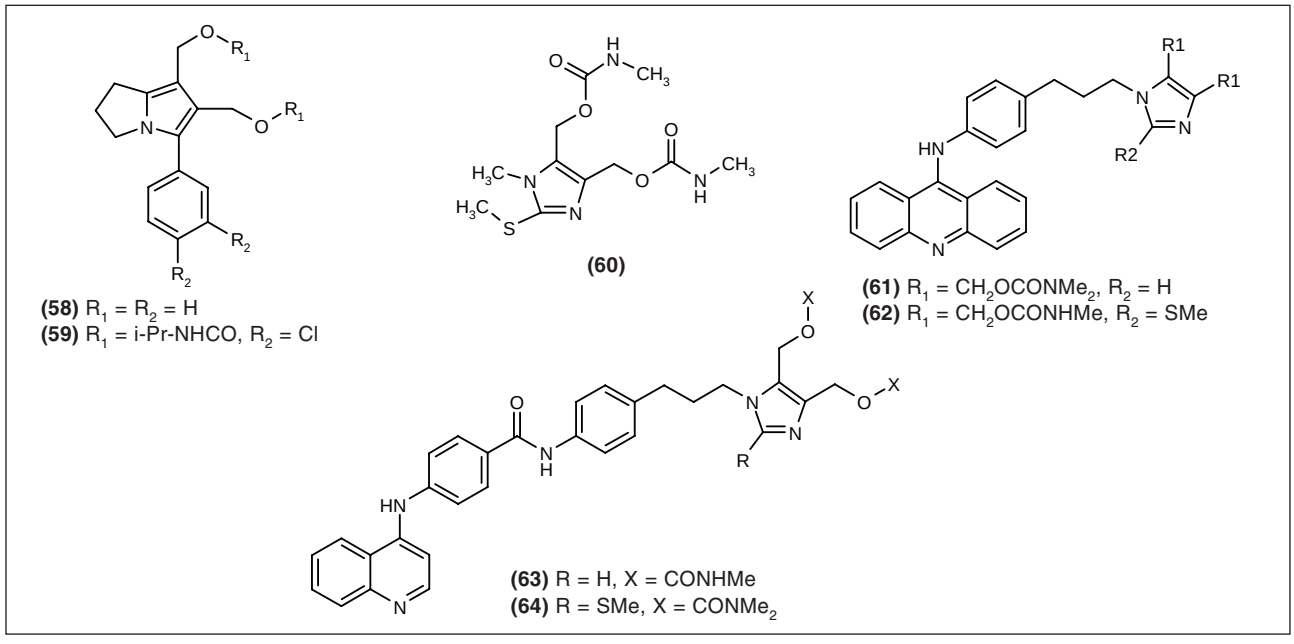
(54)
a: R = H, H
b: R = CH_2

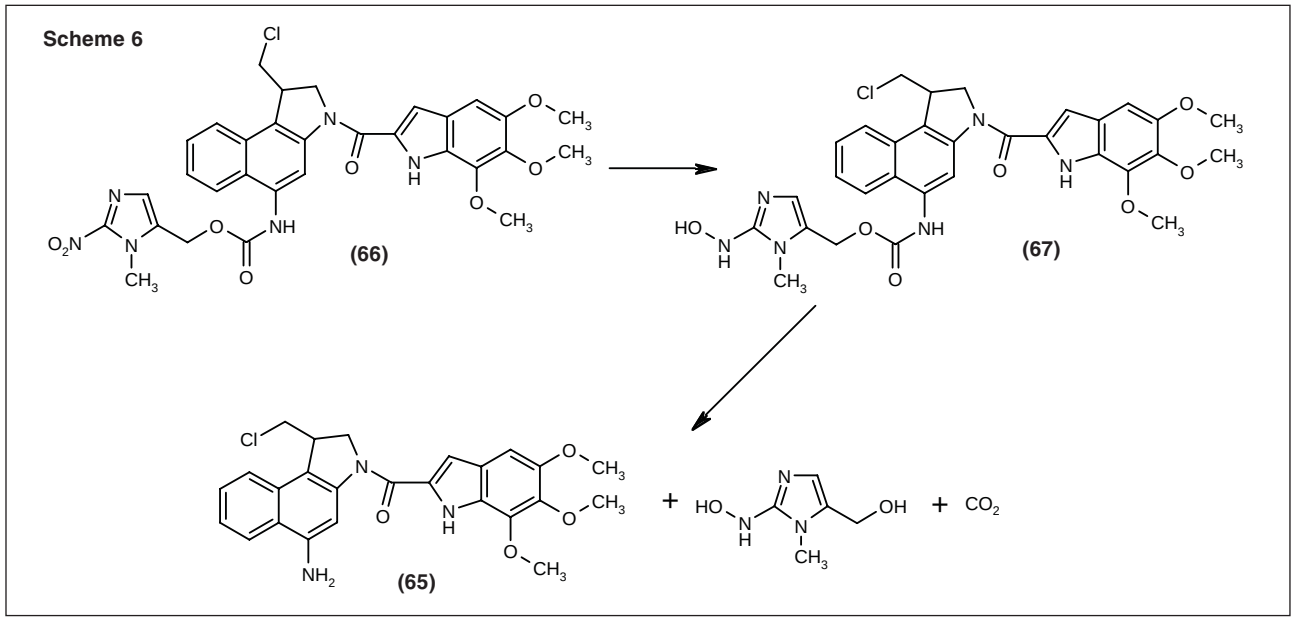


(55) R = H, H
(56) R = $CHCH_3$



(57)





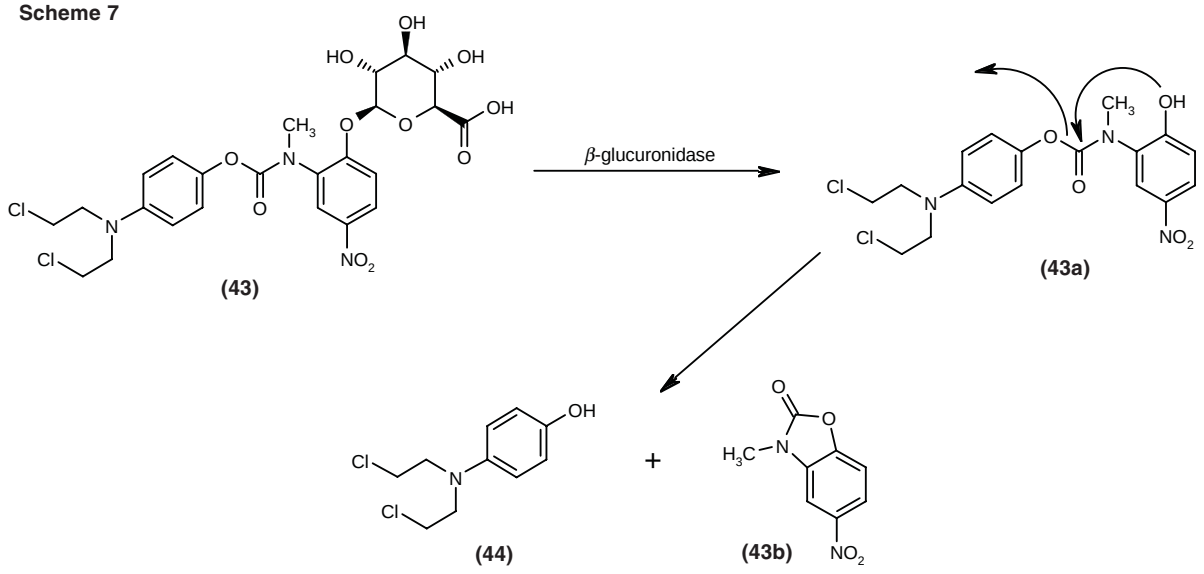
The alkylating agent **65** is a very potent cytotoxin, with IC_{50} values of 1.1 nM and 2.2 nM, respectively, against SK-OV-3 and SC3.2 cell lines. Formation of the prodrug provides 68-fold deactivation as such, while in the presence of nitroreductase and cofactor an 11-fold activation takes place.

Mechanism of action

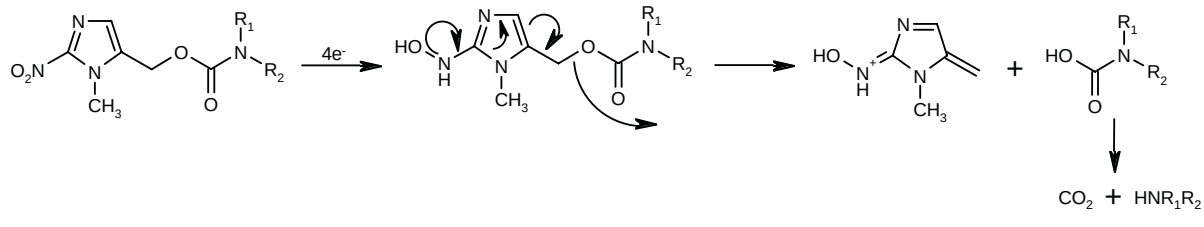
The carbamate esters developed as prodrugs of cytotoxic anticancer compounds were mainly developed with the following objectives: 1) increased solubility; 2) better taste; 3) superior pharmacological properties; 4) improved activity against multidrug-resistant tumors; 5) targeted delivery; and 6) masked toxicity.

Protection of the free phenolic and amino groups present in cytotoxic agents as their carbamate esters generally results in a significant improvement in their solubility. For example, the carbamate derivatives of 9-aminocamptothecin were 80-4,000 times more water-soluble (47), carbamate esters prepared as polyethylene glycol conjugates of cytotoxic agents showed higher water solubility (64), and about 90 times more solubility was reported for carbamate derivatives of paclitaxel (25). Conversion to carbamates also masked the disagreeable taste of amino compounds.

Scheme 7



Scheme 8



Docetaxel, the semisynthetic carbamate derivative of paclitaxel, exhibited superior pharmacological properties and improved water solubility (25). Higher potency (2-3-fold) was also observed with carbamate esters of paclitaxel against multidrug-resistant cell lines (28).

The mechanism of action of various approaches developed for targeted delivery of cytotoxic drugs to avoid side effects can be summarized as follows:

Mechanism of action of DNA-directed agents

As discussed in a previous section, the alkylating agents bis(hydroxymethyl)pyrrolizine and carmethizole, when linked to the DNA-intercalating carrier 9-anilinoacridine or the DNA minor groove-binding carrier 4-(4-quinolinylamino)benzamide, effected more efficient binding of the alkylating agents to DNA for causing cytotoxic effect (61).

Mechanism of action of enzyme prodrug therapy

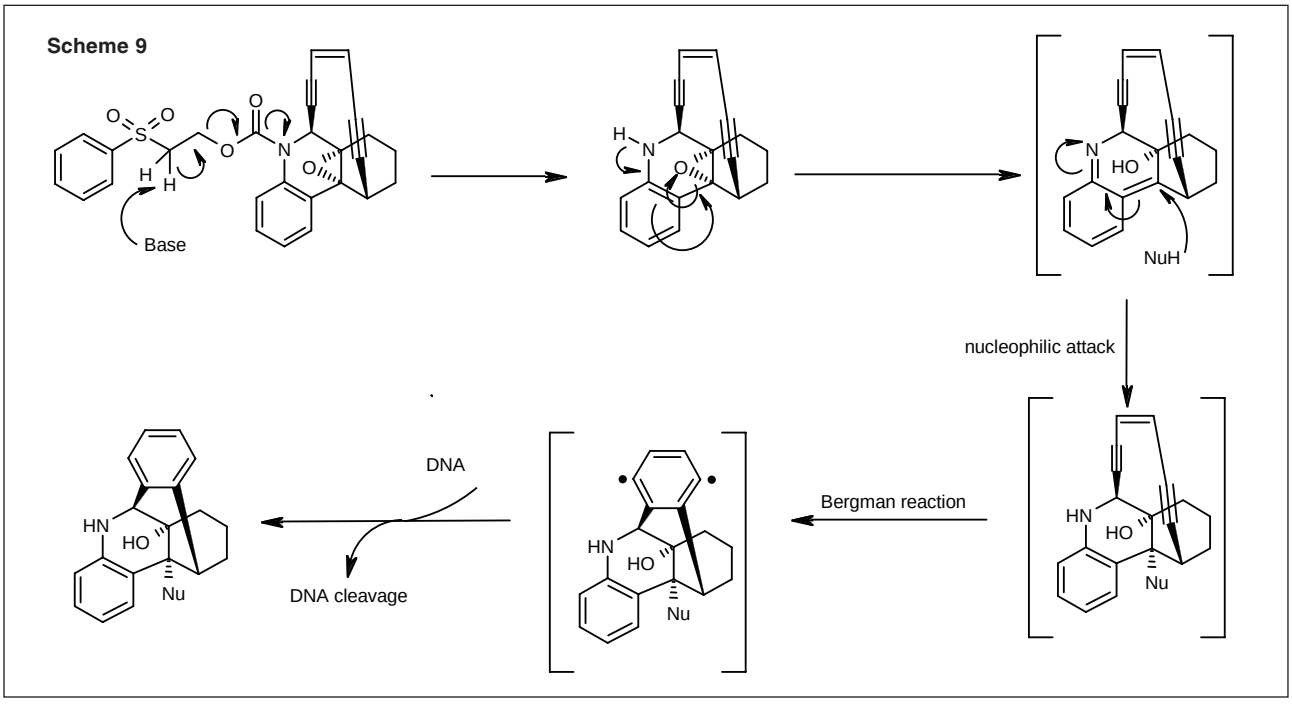
The mechanism of action of tumor-specific agents is based on the use of tumor-specific enzymes which would convert a prodrug carrying a substrate for that particular enzyme as a protecting group and release the active drug at the tumor site. Generally, the attachment of the drug at the active site to the specific substrate is through a "self-immolative" linker. This mechanism applies to prodrug monotherapy (PMT) and also to antibody-directed enzyme prodrug therapy (ADEPT). Two such mechanisms of drug release are described below.

The phenolic nitrogen mustard **44** is released from the prodrug **43** when the β-glucuronide unit is hydrolyzed under the influence of the enzyme β-glucuronidase present in the tumor cells. The intermediate product **43a** thus formed cyclizes to form the nitrobenzoxazole **43b**, with simultaneous release of the active drug **44** (Scheme 7).

Bioreduction of the nitro group to hydroxylamine by the enzyme nitroreductase takes place under hypoxic conditions present in solid tumors, but not in normal tissues, thus providing cell selectivity. The hydroxylamine product formed undergoes fragmentation to release the active drug (Scheme 8).

Mechanism of action of enediyne antitumor antibiotics

The activity of the enediyne class of compounds is due to their ability to induce DNA strand breaks. These



antibiotics contain an enediyne unit which has a tendency to undergo Bergman cycloaromatization, generating a highly reactive diradical species. When positioned properly in the minor groove of double-stranded DNA, the diradical removes a hydrogen atom at proximal deoxyribosyl sites, leading to strand scission. The enediyne structure is prevented from undergoing aromatization by the presence of the epoxide ring. An electron flow from the neighboring nitrogen causes opening of the epoxide, which brings about a conformational change resulting in Bergman cycloaromatization. This aromatization process is controlled by a carbamate residue on the nitrogen. The carbamate moiety is designed in such a manner that its removal could be effected photochemically (50), biochemically (Scheme 9) (48) or enzymatically (49) at the tumor site.

Conclusions

The use of carbamate chemistry, particularly in the design of prodrugs, has made a significant impact in the recent past. Their usefulness as protecting groups is mainly due to their optimum ability to undergo hydrolysis as compared to esters and ethers. In the present review, their use in cancer chemotherapy is described. However, there are many other areas, including bacterial and viral infections, central nervous system and cardiovascular disorders, *etc.*, where carbamates have found use, emphasizing the importance of a carbamate link, particularly in the design of prodrugs.

References

1. Plimmer, J.R. *Chemistry of pesticides*. In: Handbook of Pesticide Toxicology, Vol. 1, Pesticide Risk Characterization. Academic Press 2002.

2. Ghosh, A.K. McKee, S.P., Thompson, W.J., Darke, P.L. Zugay, J.C. *Potent HIV-1 protease inhibitors: Stereoselective synthesis of a dipeptide mimic.* J Org Chem 1993, 58: 1025-9.

3. Green, T.W., Wuts, P.G.M. (Eds.). Protective Groups in Organic Synthesis, 3rd Ed. John Wiley and Sons: Newark 1999, 505-50.

4. Raju, B., Kassir, J.M., Kogan, T.P. *Solution-phase combinatorial synthesis of ureas using nitrophenylcarbamates*. Bioorg Med Chem Lett 1998, 8: 3043-8.

5. Hutchins, S.M., Chapman, K.T. *A general method for the solid phase synthesis of ureas.* Tetrahedron Lett 1994, 35: 4055-8.

6. Agourides, C., Denis, A., Auger, J.-M. et. al. *Synthesis and anti-bacterial activity of ketolides (6-O-methyl-3-oxo-erythromycin derivatives): A new class of macrolide resistant and susceptible respiratory pathogens*. J Med Chem 1998, 41: 4080-100.

7. Bernard, N., Burgaud, B.G.M., Horwell, D.C., Lewthwaite, R.A., Martinez, J., Pritchard, M.C. *The drug design and synthesis of the high efficacy non-peptide CCK$_1$ receptor agonist PD 170292*. Bioorg Med Chem Lett 2000, 10: 1245-8.

8. Sharma, S., Abuzar, S. *The benzimidazole anthelmintics – Chemistry and biological activity.* In: Progress in Drug Research 27. Jucker, E. (Ed.). Birkhauser Verlag: Basel 1983.

9. Ingber, D., Fugita, T., Kishimoto, S., Sudo, K., Kanamann, T., Brem, H., Folkman, J. *Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumor growth*. Nature 1999, 348: 555-7.

10. *AGM-1470*. Drugs Fut 1994, 19: 981.

11. Dorey, G., Leon, P., Sciberras, S. et al. *Synthesis and antiangiogenic activity of new carbohydrate derivatives.* Bioorg Med Chem Lett 1996, 6: 3045-50.

12. Perron-Sierra, F.M., Pierré, A., Burbridge, M., Guilbaud, N. *Novel bicyclic oxazolone derivatives as anti-angiogenic agents.* Bioorg Med Chem Lett 2002, 12: 1463-6.

13. Baudoin, O., Cluvean, F., Thoret, S., Herrbach, A., Guenard, D., Cueritte F. *Synthesis and biological evaluation of A-ring biaryl carbamate analogues of rhazinilam*. Bioorg Med Res 2002, 10: 3395-400.

14. Temple, C. Jr., Rener, G.A. *Antimitotic agents: Ring analogues and derivatives of ethyl [(S)-5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-b]pyrazin-7-yl]carbamate*. J Med Chem 1992, 35: 4809-12.

15. Temple, C. Jr., Rener, G.A. *Antimitotic agents. Chiral isomers of ethyl [5-amino-1,2-dihydro-3-(4-hydroxyphenyl)-2-methylpyrido[3,4-b] pyrazin-7-yl] carbamate.* J Med Chem 1992, 35: 988-93.

16. Temple, C. Jr., Rener, G.A., Comber, R.N. *New anticancer agents: Alterations of the carbamate group of ethyl (5-amino-1,2-dihydro-3-phenylpyrido[3,4-b]pyrazin-7-yl) carbamates*. J Med Chem 1989, 32: 2363-7.

17. Temple, C. Jr., Rener, G.A. *New anticancer agent chiral isomers of ethyl 5-amino-1,2-dehydro-2-methyl-3-phenylpyrido[3,4-b] pyrazin-7-yl-carbamate*. J Med Chem 1989, 32: 2089-92.

18. Temple, C. Jr., Rener, G.A., Comber, R.N., Ward, W.R. *Antimitotic agents. Alterations at the 2,3-positions of ethyl (4-amino-1,2-dehydro pyrido [3,4-b] pyrazine-7-yl) carbamates.* J Med Chem 1991, 34: 3176-81.

19. Ram, S., Wise, A.S., Wotring, L.L., McCall, J.W., Townsend, L.B. *Synthesis and biological activity of certain alkyl-5-(alkoxycarbonyl)-1H-benzimidazole-2-carbamates and related derivatives. A new class of potential antineoplastic and antifilarial agents.* J Med Chem 1992, 35: 539-47.

20. Kumar, Y., Green, R., Borysko, K.Z., Wise, D.S., Wotring, L.L., Townsend, L.B. *Synthesis of 2,4-disubstituted triazoles as potential antitumour and antifilarial agents. I: Methyl 4-(isothiocyanatomethyl) tetrazole-2-carbamates, -selenazole-2-carbamates, and related derivatives*. J Med Chem 1999, 36: 3843-8.

21. Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P., McPhail, A.T. *Plant antitumor agent VI. The isolation and structure of Taxol, a novel antileukemic and antitumor agent from Taxus brevifolia* J Am Chem Soc 1971, 93: 2325-7.

22. Gueritte-Voegelein, F., Guenard, D., Lavelle, F., LeGoff, M.T., Mangatal, L., Potier, P. *Relationships between the structure of taxol analogues and their antimitotic activity*. J Med Chem 1991, 34: 992-8.

23. Ringel, I., Horwitz, S.B. *Studies with RP 56976 (Taxotere): A semisynthetic analog of Taxol*. J Natl Cancer Inst 1991, 83: 288-91.

24. Bissery, M.C., Guenard, D., Gueritte-Voegelein, F., Lavelle, F. *Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue*. Cancer Res 1991, 51: 4845-52.

25. Ali, S.M., Hoemann, M.Z., Aube, J., Mitscher, L.A., Georg, G.I., McCall, R., Jayasinghe L.R. *Novel cytotoxic 3'-(tert butyl) 3'-dephenyl analogs of paclitaxel and docetaxel*. J Med Chem 1995, 38: 3821-8.

26. Fang, W.S., Liu, Y., Liu, H.-Y., Xu, S.-F., Wang, L., Fang, Q.-C. *Synthesis and cytotoxicity of 2-amido docetaxel analogues.* Bioorg Med Chem Lett 2002, 12: 1543-6.

27. Zhu, J.W., Hori, H., Jajiri, H., Tsukuda, T., Taira, Z. *Synthesis and activity of brefeldin A analogs as inducers of cancer cell differentiation and apoptosis*. Bioorg Med Chem Lett 1997, 7: 139-44.

28. Ojima, I., Slater, J.C., Michand, E. et al. *Synthesis and structure activity relationship of the second generation antitumor taxoids: Exceptional activity against drug-resistant cancer cells.* J Med Chem 1996, 39: 3889-96.

29. deGroot, F.M.H., Van Berkom, L.W.A., Scheeren, H.W. *Synthesis and biological evaluation of 2'-carbamate-linked and 2'-carbonate-linked prodrugs of paclitaxel: Selective activation by the tumor-associated protease plasmin*. J Med Chem 2000, 43: 3093-102.

30. Lakey, F.N., McLeod, J.E. *The coumarins of Geijera parviflora Lindl*. Anst J Chem 1967, 20: 1943-67.

31. Simoni, D., Manfredini, S., Tabrizi, M.A., Bazanini, R. Baraldi, P.G., Baljarini, J., Clereq, E.D. *Geiparvarin analogues 2. Synthesis and cytostatic activity of 5-(4-arylbutadienyl)-3 (2H)-furanones and N-substituted 3-(4-oxo-2-furanyl)-2-buten-2- yl carbamates*. J Med Chem 1991, 34: 3172-6.

32. Brown, J.R. *Adriamycin and related anthracycline antibiotics.* Prog Med Chem 1978, 15: 125.

33. Adams, N., Blake, C., Broadhurst, M.J. et al. *Synthesis and antitumor activity of 9-[(carbamoyloxy)alkyl] anthracyclines. A novel class of anthracycline derivative*s. J Med Chem 1990, 33: 2380-4.

34. Maligres, P.E., Nicolaou, K.C., Wrasidlo, W. *A new designed tumor selective daunomycin derivative*. Bioorg Med Chem Lett 1993, 3: 1051-4.

35. Hamilton, T.C., Winker, M.A., Louie, K.G. et al. *Augmentation of adriamycin, melphalan, and cisplatin cytotoxicity in drug-resistant and sensitive human ovarian carcinoma cell lines by buthionine sulfoxime mediated glutathione depletion.* Biochem Pharmacol 1985, 34: 2583-6.

36. Izawa, T., Kato, K. *Design and synthesis of an antitumor prodrug released by the reaction with sulfhydryl compounds*. Bioorg Med Chem Lett 1995, 5: 593-6.

37. Florent, J.-C., Dong, X., Gandel, G. et al. *Prodrugs of anthracyclines for use in antibody-directed enzyme prodrug therapy*. J Med Chem 1998, 41: 3572-81.

38. Connors, T.A. *Antitumor drug with latent activity*. Biochemistry 1978, 60: 979-87.

39. Bagshawe, K.D., Springer, C.J., Searle, F. et al. *A cytotoxic agent can be generated selectively at cancer sites*. Br J Cancer 1988, 58: 700-3.

40. Senter, P.D., Saulnier, M.G., Schreiber, G.J., Hirschberg, D.L., Brown, J.P., Hellstrom, I., Hellstrom, K.E. *Antitumor effect of antibody-alkaline phosphatase conjugates in combination with etoposide phosphate*. Proc Natl Acad Sci USA 1988, 85: 4842-62.

41. Shabat, D., Lode, H.N., Pertl, U., Reisfeld, R.A., Rader, C., Lerner, R.A., Barbas, C.F. *In vivo activity in catalytic antibody*

prodrug system: Antibody catalyzed etoposide prodrug activation for selective chemotherapy. Proc Natl Acad Sci USA 2001, 98: 7528-33.

42. Schmidt, F., Monneret C. *Prodrug monotherapy: Synthesis and biological evaluation of an etoposide glucuronide-prodrug*. Bioorg Med Chem 2003, 11: 2277-83.

43. Wall, M.E., Wani, M.C., Cook, C.E., Palmer, K.H., McPhail, A.T., Sim, G.A. *Plant-antitumor agents I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from Acuminata*. J Am Chem Soc 1966, 88: 3888-90.

44. Kingsbury, W.D., Boehm, J.C., Jakes, D.R. et al. *Synthesis of water soluble (aminoalkyl) camptothecin analogues: Inhibition of topoisomerase I and antitumor activity.* J Med Chem 1991, 34: 98-107.

45. Luzzio, M.J., Besterman, J.M., Emerson, D.L. et al. *Synthesis and antitumor activity of novel water soluble derivatives of camptothecin as specific inhibitors of topoisomerase I*. J Med Chem 1995, 38: 395-401.

46. Angenault, S., Thirot, S., Schmidt, F., Monneret, C., Pfeiffer, B., Renard, P. *Cancer chemotherapy. A SN 38 (7-ethyl-10-hydroxycamptothecin) glucuronide prodrug for treatment by a PMT (prodrug monotherapy) strategy.* Bioorg Med Chem Lett 2003, 13: 947-50.

47. Leu, Y.-L., Roffler, S.R., Chern, J.-W. *Design and synthesis of water-soluble glucuronide derivatives of camptothecin for cancer prodrug monotherapy and antibody directed enzyme prodrug therapy*. J Med Chem 1999, 42: 3623-8.

48. a) Nicolaou, K.C., Dai, W.M., Tsay, S.C., Esterez, V.A., Wrasidlo, W. *Designed enediynes. A new class of DNA clearing molecules with potent and selective anticancer activity.* Science 1992, 256: 1172-8; b) Unno, R., Michishita, H., Inagaki, H. et al. *Structure-activity relationship of cyclic enediynes related to dynemicin A-I. Synthesis and antitumor activity of 9-acetoxy enediynes equipped with aryl carbamate moieties*. Bioorg Med Chem 1997, 5: 883-901; c) Unno, R., Michishita, H., Inagaki, H. et al. *Structure-activity relationship of cyclic enediynes related to dynemicin A-II. Synthesis and antitumor activity of 9- and 12-substituted enediynes equipped with aryl carbamate moieties.* Bioorg Med Chem 1997, 5: 903-19; d) Unno, R., Michishita, H., Inagaki, H. et al. *Synthesis and antitumor activity of water soluble enediyne compounds related to dynemicin A.* Bioorg Med Chem 1997, 5: 987-99.

49. a) Hay, M.P., Wilson, W.R., Denny, W.A. *A novel enediyne prodrug for antibody directed enzyme prodrug therapy (ADEPT) using E. coli B nitroreductase.* Bioorg Med Chem Lett 1995, 5: 2829-34; b) Hay, M.P., Wilson, W.R., Denny, W.A. *Nitrobenzyl carbamate prodrugs of enediynes for nitroreductase gene-directed enzyme prodrug therapy (GDEPT).* Bioorg Med Chem Lett 1999, 9: 3417-22.

50. Wender, P.A., Zercher, C.K., Beckham, S., Houbold, E.M. *A photochemically triggered DNA cleaving agent: Synthesis, mechanistic and DNA cleavage studies on a new analog of the antitumor antibiotic dynemicin*. J Org Chem 1993, 58: 5867-72.

51. Wender, P.A., Beckham, S., O'Leary, J.G. *A second generation photochemically activatable dynemicin analog: A concise synthesis and DNA cleavage studies.* Synthesis 1994, 1278-82.

52. Dowell, R.I., Springer, C.J., Davies, D.H. et al. *New mustard prodrugs of antibody directed enzyme prodrug therapy: Alternative to the amide link*. J Med Chem 1996, 39: 1100-5.

53. Schmidt, F., Florent, J.C., Monneret, C., Straub, R., Czech, J., Gerken, M., Bosslat, K. *Glucuronide prodrugs of hydroxy compounds for antibody directed enzyme prodrug therapy: A phenyl nitrogen mustard carbamate.* Bioorg Med Chem Lett 1997, 1: 1071-6.

54. Papot, S., Comband, D., Bosslet, K., Gerken, M., Czech, J., Gesson, J.-P. *Synthesis and cytotoxic activity of a glucuronylated prodrug of nor nitrogen mustard*. Bioorg Med Chem Lett 2000, 10: 1835-7.

55. Hay, M.P., Wilson, W.R., Denny, W.A. *Design synthesis and evaluation of imidazolyl methyl carbonate prodrugs of alkylating agents*. Tetrahedron 2000, 56: 645-57.

56. Jordan, A.M., Khan, T.H., Osborn, H.M.I., Photion, A., Riley, P.A. *Melanocyte-directed enzymes prodrug therapy (MDEPT): Development of a targeted treatment for malignant melanoma.* Bioorg Med Chem 1999, 1: 1775-80.

57. Reynold, R.C., Tiwari, A., Harwell, J.E. et al. *Synthesis and evaluation of several new (2-chloroethyl) nitrosocarbamates as potential anticancer agents*. J Med Chem 2000, 43: 1484-8.

58. Sagnon, M.J., Howard, P.W., Gregson, S.J., Eno-Amooquayl, E., Burke, P.J., Thurston, D.E. *Design and synthesis of novel pyrrolobenzodiazepine (PBD) prodrugs for ADEPT and GDEPT*. Bioorg Med Chem Lett 2000, 10: 2083-6.

59. Elliot, W.L., Fry, D.W., Anderson, W.K., Nelson, J.M., Hook, K.E., Howkins, P.A., Leopold, W.R. *In vivo and in vitro evaluation of the alkylating agent carmethizole*. Cancer Res 1991, 51: 4581-7.

60. Atwell, G.J., Fan, J.-Y., Tan, K., Denny, W.A. *DNA directed alkylating agents 7. Synthesis, DNA interaction and antitumor activity of bis (hydroxymethyl) and bis(carbamate)-substituted pyrrolizines and imidazoles*. J Med Chem 1998, 41: 4744-54.

61. Atwell, G.J., Tercel, M., Boyd, M., Wilson, W.R., Denny, W.A. *Synthesis and cytotoxicity of 5-amino-1-(chloromethyl)-3-[5,6,7-(trimethoxy indole-2-yl)carbonyl]-1,2-dihydro-3H-benz[e]indole (amino-seco-CBI-TMI) and related 5-alkylamino analogues: New DNA minor groove alkylating agents*. J Org Chem 1998, 63: 9414-20.

62. Hay, M.P., Sykes, B.M., Denny, W.A., Wilson, W.R. *A 2-nitroimidazole carbamate prodrug of 5-amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3H-benz[e] indole (amino-seco-CBI-TMI) for use with ADEPT and GDEPT.* Bioorg Med Chem Lett 1999, 9: 2237-42.

63. Wardman, P., Clarke, E.D. *The role of catalytic superoxide formation in the O_2 inhibition of nitroreductase.* Biochem Biophys Res Commun 1975, 67: 1267-74.

64. Greenwald, R.B., Pendri, A., Conover, C.D. et al. *Drug delivery systems employing 1,4- or 1,6-elimination: Poly(ethylene glycol) prodrugs of amine containing compounds.* J Med Chem 1999, 42: 3657-67.