# Distamycins: Strategies for Possible Enhancement of Activity and Specificity

Rita Cortesi* and Elisabetta Esposito

*CoReS Techno Group, Department of Pharmaceutical Sciences, University of Ferrara, BioPharmaNet, 44121 Ferrara, Italy*

**Abstract:** The present review focused on the strategies aimed to possibly solve toxicity problems of distamycins. Distamycins are compounds characterized by an oligopeptidic pyrrolocarbamoyl frame ending with an amidino moiety. This class of compounds displays antiviral and antibiotic activity and shows interesting antiprotozoal activity related to the ability to reversibly bind to the minor groove of DNA with a high selectivity for TA-rich sequences. In consideration of their potential therapeutic properties, the synthesis of new distamycin derivatives and especially the development of controlled delivery strategies, could lead to important advantages in the clinical use of these molecules, possibly overcoming or mitigating the low solubility, specificity and toxicity problems associated with their use.

To these aims an ensemble of the main synthetic distamycin derived compounds and of the potential drug delivery systems for distamycins described in literature is reviewed.

**Keywords:** Liposomes, distamycins, antiproliferative activity, antitumor drugs.

## DISTAMYCIN A AND RELATED COMPOUNDS: ADVANTAGES AND DISADVANTAGES

Distamycin A (Fig. **1A**) is a naturally occurring antibiotic agent isolated in 1962 from the cultures of *Streptomyces distallicus* [1], then obtained by synthesis in solution [2] and on solid phase [3], characterized by interesting antibacterial and antiviral activities. The most important antiviral effects are directed against DNA-containing viruses, such as herpes simplex, herpes zoster and vaccine virus, while no activity is evident toward RNA viruses [4]. In addition, distamycin A was found to show antiprotozoal activity against *Plasmodium falciparum* [5].

The oligopeptidic pyrrolecarbamoyl frame ending with an amidino moiety of distamycin A allows the reversibly bind to DNA minor groove *via* hydrogen bonds, Van der Waals and electrostatic interactions. Hydrogen bonding between the groove floor base pairs and the linking amides, together with the electrostatic stabilizing interactions to the protonated amines, are primary contributors to the overall ligand/DNA stability. Particularly, a strong preference of DNA minor groove with adenine-thymine (AT) rich sequences occurs [6-8] probably because the absence of the protruding 2-amino group of guanine (G) and the deeper and narrower AT minor-groove is optimal for accommodating the shape of the compound and for maximizing van der Waals contacts.

As a result of DNA minor groove occupation, distamycin A is a potent inhibitor of Werner and Bloom syndrome helicases [9], of the catalytic activity of topoisomerases I and II [10-12] and the transcription of RNA polymerase II [13-15]. Distamycin A has also been used to study human fragile sites, linked to the incidence of cancers and other severe disorders.

The stoichiometric ratio between distamycin and DNA can be 1:1 or 2:1 depending on the DNA sequence. Particularly, distamycin will bind 1:1 minor groove binding within DNA sequences with four to five successive AT base pairs. On the other hand, the sites having five successive AT or one guanine-cytosine (GC) and four AT base pairs can accommodate two distamycin molecules stacked head to tail with charged groups located at opposite ends.

Much of what is known about the way in which minor groove binding compounds interact with DNA is based on the studies of the distamycin A and netropsin (Fig. **1B**) [16]. Distamycin A and netropsin have served as the basis for the development of "synthetic programmable oligomers", that are molecules made up of functional groups able to read individual Watson–Crick base pairs in the order by which these functional groups are joined together [17-18]. The resulting DNA binding polyamides have allowed the very specific targeting of defined sequences within the genome, such as transcription factor binding sites. These compounds are being exploited for many reasons, including their ability to interfere with normal DNA/protein interactions, the identification of particular DNA sites within cells, such as sequence polymorphisms, and their ability to direct DNA binding of artificial transcription factors.

Distamycin A and netropsin were also used as DNA minor groove sequence-selective vectors of alkylating functions. A number of X-ray crystallographic and NMR studies have shown that these molecules bind into the minor groove of B-type DNA duplexes with high selectivity for AT-rich sequences. Van der Waals forces and hydrogen bonding play the key role for the DNA binding, whereas

*Address correspondence to this author at the CoReS Techno group, Department of Pharmaceutical Sciences, University of Ferrara, BioPharmaNet, 44121 Ferrara, Italy; Tel: +390532455257; Fax: +390532455953; E-mail: crt@unife.it

1389-5575/10 $55.00+.00 

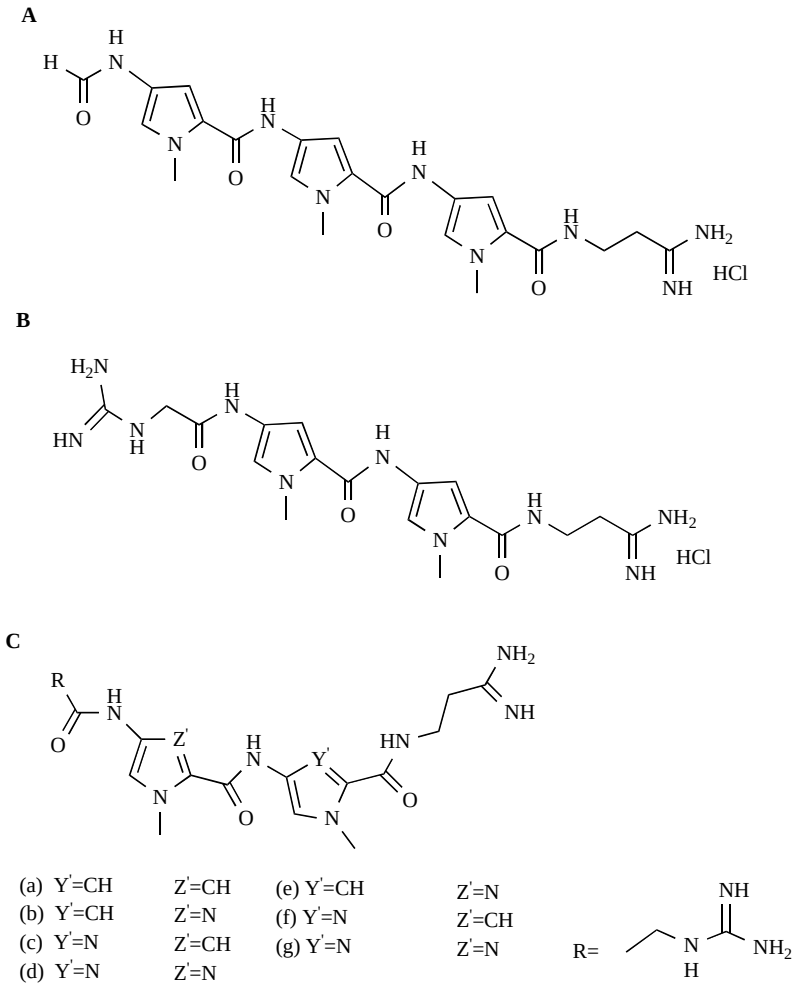



**Fig. (1).** Chemical structure of distamycin A (**A**), netropsin (**B**), lexitropsins (**C**).

hydrophobic interactions and electrostatic binding component from the cationic end stabilize the complex [19-21].

As reported by literature, distamycins display very interesting possibilities to be employed as anticancer drugs [7,9-13,16,22]. In addition, distamycin A has driven researcher's attention not only for the biological activity, but also for its non-intercalative binding to the minor groove of double-stranded B-DNA. The pyrrole-amide skeleton of distamycin A has been also used as DNA sequence selective vehicles for the delivery of alkylating functions to DNA targets, leading to a sharp increase of its cytotoxicity. In the last years, several hybrid compounds, in which some antitumor derivatives or active moieties of known antitumor agents have been tethered to distamycin frames, have been designed, synthesized and tested [7]. Several efforts have been made to modify DNA sequence selectivity and stability of the distamycin A based on structural modifications, such as the replacement of pyrrole by other heterocycles and/or benzoheterocycles, obtaining a novel class of minor groove binding molecules called "lexitropsins" (Fig. **1C**). The role of the amidino moiety, by means of the substitution with various groups, which includes ionizable, acid or basic, and non-ionizable groups, has been also studied. Other structural variant of distamycin A made up of a polyamide chain and an alkylating group are called "combilexins" [23].

Moreover, in consideration of their potential therapeutic properties, the synthesis of new distamycins and especially the development of controlled delivery strategies, could lead to important advantages in the clinical use of these molecules possibly overcoming or mitigating the solubility, specificity and toxicity problems associated with their use [24-25].

In this review will be considered several classes of distamycins reported in the published literature. In addition, some delivery systems for possible distamycins' administration will be here described. These delivery systems are proposed since it is known that they (a) can enhance drug cellular internalization, (b) can generally decrease unwanted systemic toxic effects and (c) may increase drug solubility in biological fluids, modulating at the same time the drug release profile. Furthermore, in some cases, the use of phospholipid vesicles may have the further advantage of leading to specific release by passive or active targeting strategies [26].

## TRYING TO SOLVE THE PROBLEMS

### 1. Synthesis of New Derivatives

It is well known that distamycin A is a good antibiotic agent active against some viruses, Gram-positive bacteria

and protozoa, even if it is quite inactive as antitumor agent [1]. During the past few years, studies have indicated that the antitumor activity of DNA-binding drugs is not due to an interaction with DNA but can be attributed to the inhibition of enzymes that regulate DNA topology. However, in order to increase the antitumor activity and at the mean time the DNA binding affinity of distamycins, a number of natural and synthetic molecules have been proposed [21-22,24]. The synthesis of new distamycin derivatives could lead to important advantages in the clinical use of these molecules possibly overcoming many problems associated with their use, such as solubility, specificity and toxicity [24-25].

Studies on structure-activity relationship (SAR) demonstrated that the sequence specificity and high affinity of distamycins derives from a combination of many interactions, such as van der Waals contacts, hydrogen bonding and electrostatic interactions of the cationic amidine side chain with the phosphate backbone of DNA [6].

The structural modifications considered in the present review to possibly improve the biological activity of distamycins are generally schematized in Fig. (**2**).

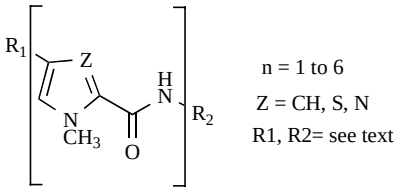



**Fig. (2).** General scheme of structural modifications of distamycin A considered in the present review.

Particularly, the number of pyrrole units, the presence of more than one heteroatom within the pyrrole unity, the saturation of the eterocycles and the presence of different acyl chain residues both at the N- and C-terminus have been investigated [16-25,27-75, 115].

To deeper understand structure-activity relationships of the distamycin analogues, Khedkar *et al*. performed a 3D-QSAR analysis using comparative molecular field analysis (CoMFA) to map the structural features contributing to the inhibitory activity of these molecules [27]. Three CoMFA models, each using activity data against drug-resistant bacterial strains, namely methicillin-resistant *Staphilococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-susceptible *Enterococcus faecalis* (VSEF), were considered showing a good correlation and predictive power. It was found that molecules with an electropositive substituent on the concave surface facing DNA upon binding, and an electronegative substituent on the opposite side (the convex surface) at the R1 substitution of the distamycin skeleton would exhibit good inhibitory activity and also contribute to their broad-spectrum activity.

The synthesis of different analogues of distamycin A allows the establishment of some important molecular requirements for bioactivity. For instance, it was observed that the saturation of distamycin pyrrole rings determines a high reduction of the DNA binding affinity, thus the new derivative structure must maintain the unsaturated N-methyl pyrrole rings [28].

Many studies demonstrated that it is possible to achieve high affinity and selectivity of DNA binding by the distamycin A analogue containing an isopropyl-substituted thiazole in place of one of the *N*-methylpyrroles. This derivative is selective for the sequence 5′-ACTAGT-3′ to which it binds with high affinity [24]. Moreover, it was found that a tris-pyrrole analogue of distamycin A lacking the N-terminus formamide unit does not affect the ability of DNA to bind to AT-rich sequences [29-30]. The synthesis of this compound has suggested that this function can be dispensed without affecting the DNA-binding properties to AT-rich sequences. However, in the absence of H-bond donor or acceptor at the N-terminus, a minimum of three pyrrole carboxamide units is necessary for the onset of DNA binding. On the other hand, the tetra-pyrrole distamycin A derivative (Fig. **3A**) is characterized by a very low cytotoxic effect on L1210 leukemia cell line but it demonstrated an approximately 20-fold higher activity with respect to distamycin A [16]. Thus, the higher the number of pyrrole units in the oligopeptidic frame, the higher the sequence specificity for longer tracts of AT-rich DNA, as a result of the greater availability of hydrogen bonding and van der Waals surface. [31].

Tetra-amide derivatives presenting lipophilic alkyl substituents on the pyrroles, displayed strong antibacterial activity against antibiotic resistant strains of *Staphylococcus aureus* and *Enterococci* [32]. Among the evaluated N-substituents, it was found that isoamyl and cyclopropyl-methyl groups led to the most active compounds with minimum inhibitory concentrations in the submicromolar range. Tetra-amides carrying a methyl-pyrrole did not show antibacterial activity [33]. The five-pyrrole distamycin A derivative acts as a telomerase inhibitor. Exposure of human melanoma cell extracts to this compound induced a dose-dependent inhibition of telomerase activity. The increase of the number of pyrrolic rings over six units produced molecules being out of phase with DNA.

Moreover, several bis-distamycin derivatives (obtained by linking two symmetrical deformyldistamycin residues to a dicarboxylic acid derivative) demonstrated higher antiretroviral activity, especially against Human Immunodeficiency Virus (HIV), with respect to that shown by distamycin A [34-35]. For instance, NSC 651016 (Fig. **3B**) is able to selectively inhibit chemokine receptors involved in the entrance of HIV-1 into human cells. In this view, NSC 651016 could be an attractive candidate as chemotherapeutic treatment as well as to prevent HIV-1 sexual transmission [36].

Other compounds characterized by the presence of one to four thiazole units and dimethylaminopropyl or EDTA moieties on the C-terminus (see general formula on Fig. **3C**) showed an higher activity with respect to distamycin A in inhibiting HIV-1 reverse transcriptase [37].

In addition, the three N-methyl pyrrole carboxamide units of distamycin A linked at the N-terminal position to an isothiazole ring lead to the production of various compounds (see general formula in Fig. **3D**) showing excellent activity against a broad spectrum of Gram-positive bacteria [38].

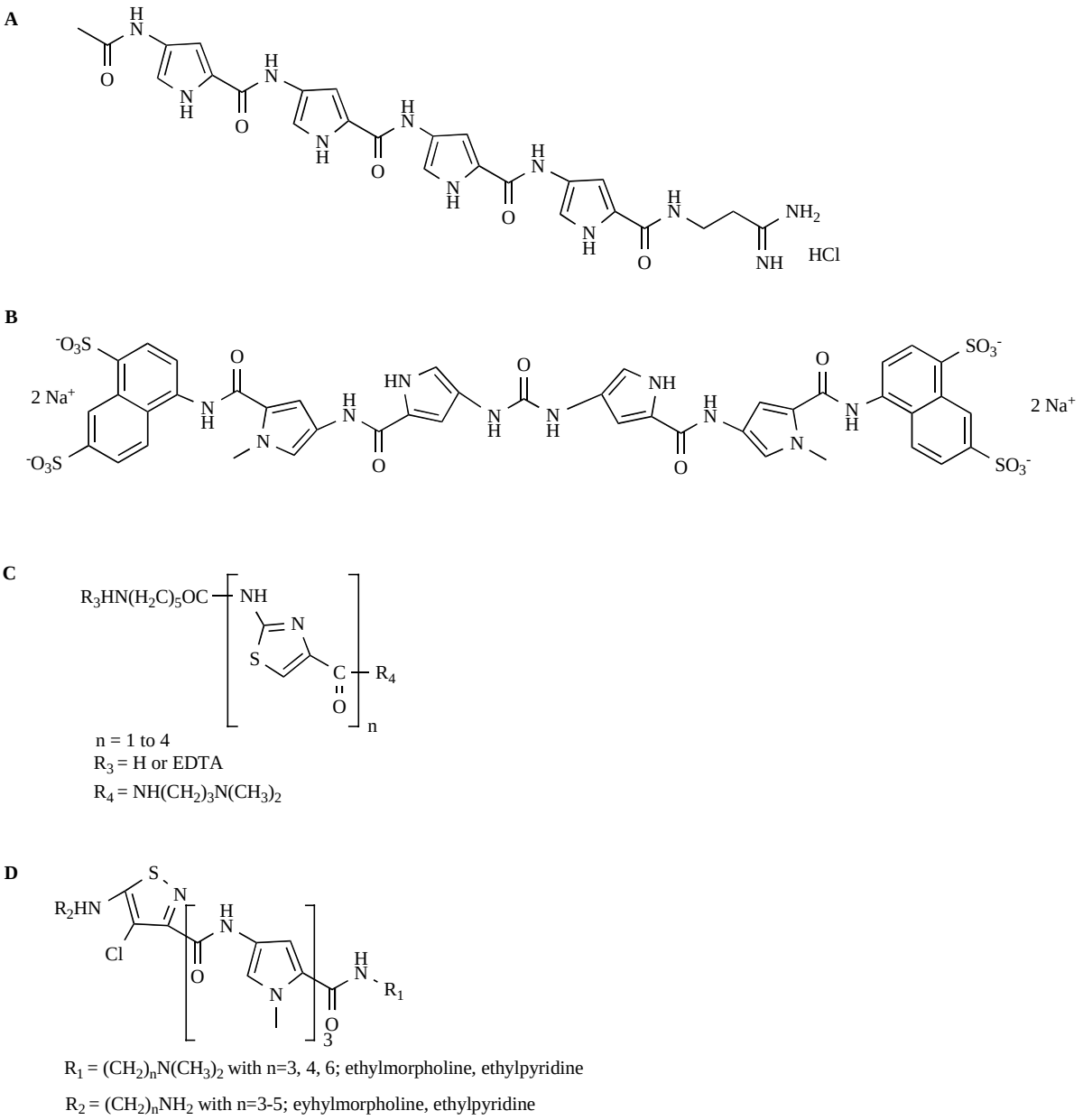



**Fig. (3).** Chemical structure of tetrapyrrole distamycin (**A**), NSC651016 (**B**), general formula of distamycin thiazole derivatives (**C**) and general formula of N-terminal isothiazole compounds (**D**).

### *A. Benzoic Acid Mustard Distamycins*

The groups of Arcamone and D'Alessio [39-40] synthesized distamycins substantially more cytotoxic than distamycin A, in which the formyl group has been substituted by benzoyl nitrogen mustard (BAM), nitrogen mustard, halogenoacryloyl and epoxy carbonyl moieties. Halogenoacrylic derivatives are peculiar compounds being characterized by a moiety (i.e. α-bromoacrylic acid) not *per se* cytotoxic but able to interact with biological nucleophiles. It has to be underlined that also for BAM derivatives, the hig\her the number of pyrrole rings, the higher are both the *in vitro* cytotoxicity and the *in vivo* potency on L1210 murine leukemia cells. Particularly, PNU151807 (Fig. **4A**) is the most active compound being approximately 1000-fold more potent than distamycin A [41-42]. Preclinical studies revealed that PNU 151807 possesses a broad spectrum of activity.

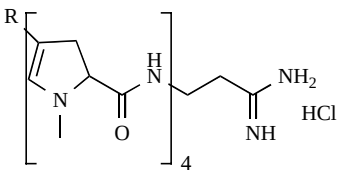
The structure–activity relationship of distamycins characterized by the presence of different halogenoacrylic moieties and modified have been investigated by Cozzi et al. [43]

It was found that both α-bromine and chlorine-acrylic derivatives display a high cytotoxicity whereas α-fluoroacylic and acrylic derivatives are characterized by a low activity.

The acryloyl-substituted distamycins, such as brostallicin (Fig. **4B**) and PNU-151807 are typified by the presence of ionizable or non-ionizable groups instead of the typical amidino moiety. For the α-bromoacrylamido derivatives, the presence of non-basic groups, such as amidoxime or

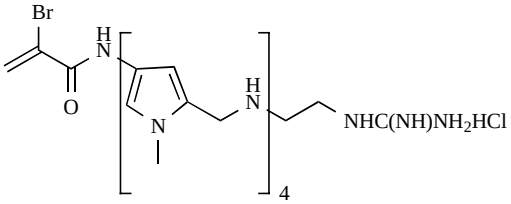
cyanoamidine, led to compounds able to maintain or even increase the cytotoxicity of the parent amidino derivative [44].

**A**

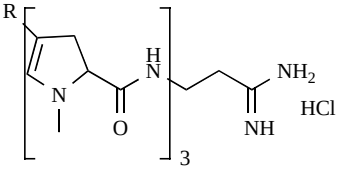



R = $CH_2$=CBrCONH

**B**




**C**




R = 4-[$(ClCH_2CH_2)_2N$]$C_6H_4$CONH

**Fig. (4).** Chemical structure of BAM distamycins: PNU151807 (**A**), brostallicin (**B**) and tallimustine (**C**).

In this series, the most important compounds are represented by the α-halogenoacrylic derivatives. Particularly, the α-bromoacrylamido derivative brostallicin was found active *in vitro* and *in vivo* against a broad spectrum of tumor cell lines and tumor xenografts with an improved therapeutic window over other alkylating agents, such as bizelesin, adozelesin and tallimustine [45]. Based on these results, brostallicin has been selected for the phase I clinical development.

As previously stated, analogues of distamycin A containing many pyrrole units have greater specificity for longer AT tracts due to an increase in hydrogen bonding and van der Waals surface contacts [6-15]. Distamycin A and its four-pyrrole homologue have been used as the DNA sequence selective targeting agents to create many synthetic compounds containing an alkylating group [42]. Particularly, the formyl group of distamycin was changed with benzoyl nitrogen mustard or chlorambucil and epoxycarbonyl moieties [16] obtaining compounds considerably more cytotoxic than the two components [8, 16].

The BAM derivative of distamycin A, tallimustine (Fig. **4C**) was selected as an antineoplastic drug candidate in view of its high activity against a wide variety of murine tumors and human xenografts and expressed a promising antitumor activity both *in vitro* and *in vivo* [46]. The cytotoxicity of tallimustine is related to the ability to form interstrand cross-links in DNA that allows the inhibition of DNA replication and transcription. On the other hand the antitumor activity of tallimustine may be due to the hindrance of the binding of some transcription factors to their DNA consensus sequences. Unfortunately, tallimustine showed a severe myelotoxicity that probably impaired the achievement of effective therapeutic doses and its phase II clinical development was discontinued [47-50].

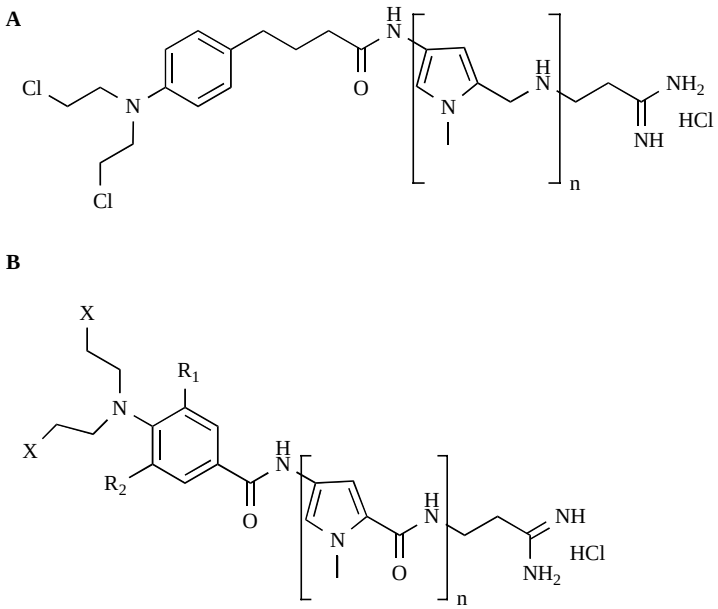
It is remarkable that cytotoxicity can be improved by increasing the number of pyrrole residues from three to four [39-40]. Moreover, the substitution of the BAM with the chlorambucil moiety gave the derivative MEN-10710 (Fig. **5A**), endowed with greater cytotoxicity, *in vivo* higher potency and reduced myelotoxicity with respect to tallimustine. MEN-10710, differently from tallimustine, is able to form DNA double-strand cross-links and to induce DNA breaks in the same way of classical nitrogen mustard [51]. The carbocyclic analogue of MEN-10710, in which pyrroles were substituted with phenyl rings, showed very low antiproliferative activity (90–100 μM) against MCF-7 mammalian tumor cells, with an increased affinity toward GC sequences [52].

Bielawsky and Bielawska synthesized and studied the structure-activity relationship of carbocyclic analogues of netropsin and distamycin A [47]. It is worth noting that the carbocyclic analogues of netropsin and distamycin are readily available, easily modifiable and stable under most experimental conditions. It was found that the obtained compounds bind to AT sequences more weakly than netropsin and distamycin. However, these compounds showed sequence selectivity and cytotoxic effects in cultured breast cancer MCF-7 cells, but did not show significant advantages in terms of activity compared to chlorambucil [47].

In a study on chemical and biological activities of alkylating agents, Bardos [53] has shown that in aromatic mustards, the reactivity is largely controlled by the leaving group ability of the halogen and that the replacement of chlorine with bromine greatly increased the antitumor activity *in vivo* against Walker carcinosarcoma 256 [54]. In this view, the effects of the replacement of the bis(2-chloroethyl) aniline on tallimustine with bis(2-haloethyl) aniline groups was studied, obtaining distamycins with general formula reported in Fig. (**5B**).

Comparing the activity of tallimustine both *in vivo* and *in vitro* on L1210 cell line with that reported for its ortho-methyl and diortho-methyl analogues, it was confirmed that *in vivo* antileukemic activity is poorly correlated with cytotoxicity. Indeed ortho-methyl and diortho-methyl analogues of tallimustine showed very good antileukemic activity (higher than that reported for tallimustine), but almost 4- and 70-fold lower cytotoxicity, respectively [55].

In order to obtain the increase of both the mustard reactivity and the distance between the alkylating moiety and the DNA-binding distamycin frame, a new class of distamycin derivatives, of general formula reported in Fig. (**5C**), was investigated. Particularly, in this class of compounds the distamycin's formyl group was substituted by an alkyl and/or alkoxy substituted cinnamoyl moiety bearing as alkylating group an N-(halo) alkyl-N-haloethyl-amino group in which the amidino moiety has been replaced by moieties of different physico-chemical features [56].




n = 2-4; X= OH, F, Cl, Br or I

$R_1 = R_2$ or $R_1$ different from $R_2$, wherein R and $R_1$ equal to H, Me or Et.

n = 2-4; X= OH, F, Cl, Br or I

$R_0$ = alkyl or $CH_2CH_2X$

$R_1 = R_2$ or $R_1$-$R_2$, with R and $R_1$ equal to H, alkyl, alkoxy or halogen.

B = $C(NH)NH_2$, imidazolin$^{-2}$-yl, imidazol$^{-2}$-yl, $C(NCN)NH_2$, $C(NOH)NH_2$, $C(NNH_2)NH_2$, $NHC(NH)NH_2$, CN, $CONH_2$.

**Fig. (5).** Chemical structure of MEN10710 (**A**), general formula of bis(2-haloethyl) aniline distamycins (**B**) and general formula of alkyl and/or alkoxy substituted cinnamoyl distamycins (**C**).

Among these derivatives, the cinnamic nitrogen mustard PNU-157911 (Fig. **6A**), due to its increased chemical reactivity, is considerably more potent both *in vivo* and *in vitro* against L1210 leukemia than tallimustine itself [55-56]. On the other hand, the ethyl-chloroethyl half-mustard PNU-160366 (Fig. **6B**) showed cytotoxicity substantially equivalent PNU-157911, although it was less potent *in vivo*. PNU-157911 and PNU-160366 show a cytoxicity/myelotoxicity ratio significantly improved as compared to tallimustine, and for this reason they have been selected for further extensive evaluation on murine solid tumors and human xenografts [55].

The potentiality of one-arm mustard derivatives of distamycin A as cytotoxic agents has been confirmed by the synthesis of sulphur mustard derivatives of distamycin. Indeed, the cinnamoyl sulphur mustard derivative PNU-193821 (Fig. **6C**) is characterized by a potent cytotoxic activity against L1210 leukemia cells, being one order of magnitude more potent than PNU-157911 and more than 60-fold more potent than tallimustine [57].

Tallimustine and PNU-157911 were modified in the amidino moieties obtaining a series of compounds with successful improvement of the biological activities. Particularly, the BAM derivatives cyanoamidine and N-methyl-amidine, containing respectively a weak and a strongly basic amidine-like moiety, maintains cytotoxic activity both *in vitro* and *in vivo* comparable to that of tallimustine. On the other hand, the low cytotoxicity of amidrazone indicates that the presence of an amidino-like structure does not guarantee significant activity [58]. Among non-amidine-like derivatives, it has to be underlined the low cytotoxicity and *in vivo* activity against L1210 cells of the tallimustine derivative in which the propion-amino moiety has been replaced by a dimethyl-aminopropyl group [59]. Otherwise, tallimustine

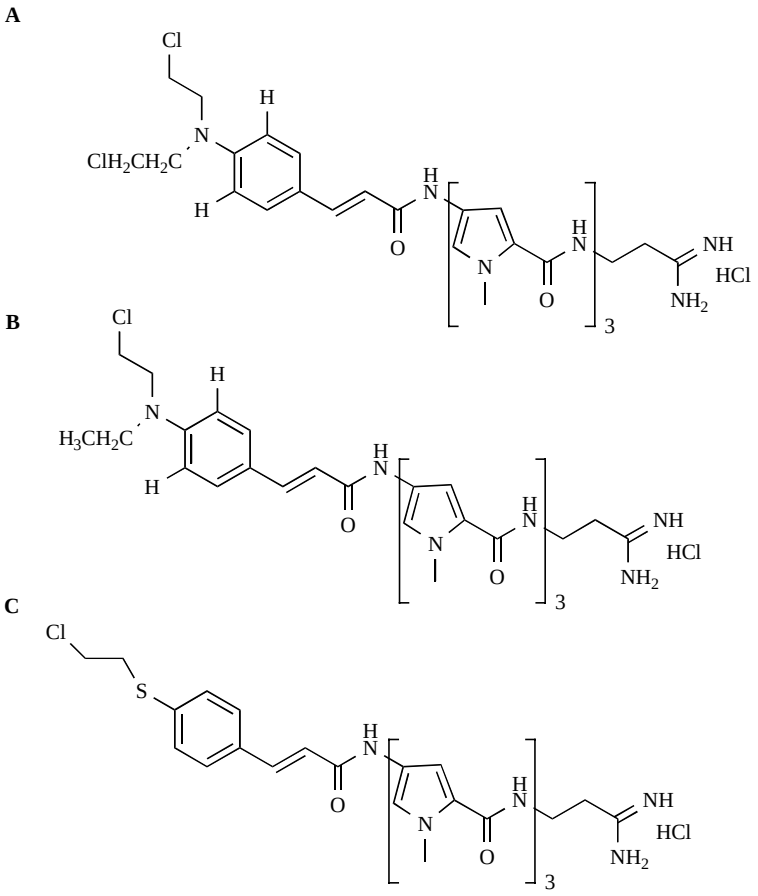



**Fig. (6).** Chemical structure of PNU157911 (**A**), PNU160366 (**B**) and PNU193821 (**C**).

and this last derivative showed the same cytotoxicity when tested against human erythroleukemic K562 cells [60].

Taken together these results suggested that the presence of a basic moiety is not an absolute requirement for *in vitro* and *in vivo* activity of distamycin mustard derivatives, thus contrasting with the opinion that an electrostatic interaction between the cationic moiety of the compound and the negatively charged phosphate residues of DNA is one of the main contributions to molecular recognition of distamycins.

### *B. Heterocyclic Analogues of Tallimustine*

As a consequence of what above reported, many researchers performed the synthesis of distamycins carrying other types of heterocyclic rings instead of pyrroles [61-63]. Particularly, the synthesis and antitumor activity of tallimustine analogue bearing all the possible pyrrole–pyrazole combinations have been investigated in order to find a possible relationship between the number of pyrazoles, their position and the cytotoxic activity [64-65].

The increased number of pyrrole units in the oligopeptidic frame led to an increase of cytotoxicity. Nevertheless, the increase of pyrrole units led to an increase of the *in vivo* potency, although failed to improve antileukemic activity. On the contrary, the pyrazole homologue showed a 3-fold less potent antiproliferative effect as compared to that of the parent compound [61-62]. The tripyrazole derivative showed a cytotoxic activity *in vitro* almost 20-fold lower with respect to the tripyrrole counterpart tallimustine and substantially devoid of antileukemic activity *in vivo*.

Among the synthesized hybrids pyrrole–pyrazole, the two isosters of tallimustine carrying pyrazole rings instead of the pyrrole near the amidine terminus and the central pyrrole, showed cytotoxicity towards L1210 cells comparable to that of tallimustine [6,63-65]. The compounds displaying pyrazole rings instead of one or two pyrrole units near the BAM moiety did not show activity [65].

The antileukemic activity of these pyrrole–pyrazole analogues of tallimustine strictly depends on the positions of pyrazole and pyrrole ring. Particularly, the antileukemic effect appeared lower when pyrazole was close to alkylating moiety. On the other hand, the presence of a pyrrole ring nearby BAM moiety was essential to obtain both *in vitro* and *in vivo* activities.

The crucial role of pyrrole ring close to the BAM has been also confirmed by the synthesis of two tallimustine analogues in which this heterocycle was replaced by imidazole [66-67] or thiazole [63]. This replacement transforms the steric interference between the compound and DNA into an energetically favorable hydrogen bond. For this reason, a novel class of minor groove binding molecules, called ‘‘lexitropsin’’ (Fig. **1C**), has yielded ligands with an increased tolerance for GC base pairs at their binding sites. Otherwise, among the derivatives bearing the pyrazole in the same position, the thiazole and imidazole tallimustine

derivatives were devoid of cytotoxicity being *in vitro* 700-, 30-, 40-fold less active than tallimustine, respectively [67-68].

With the aim to obtain new oligopeptides structurally related to distamycin but bearing both mixed heterocyclic moieties and a nitrogen mustard moiety as alkylating agent, the synthesis of a series of hybrid pyrrole–imidazole distamycin analogues, has been reported [69]. It was found that the lexitropsin mustards are characterized by a lower cytostatic activity (around 10–30-fold) with respect to the mustard derivative obtained from distamycin against the KB cancer cell line [70].

As previously reported, the type of alkylating moiety heavily influences the cytotoxicity of compounds carrying the same oligopeptidic chain [68]. *In vivo* and *in vitro* studies on L1210 murine tumor showed that the di-bromine nitrogen mustard PNU 157977 (Fig. **7A**) is approximately 40-fold more cytotoxic than the di-chlorine counterpart [69]. The same compound with a single dose of 1.56 mg/kg caused an increased survival time, higher 5- and 3-fold with respect to that of tallimustine and bis(2-chloroethyl) aniline derivative, respectively.

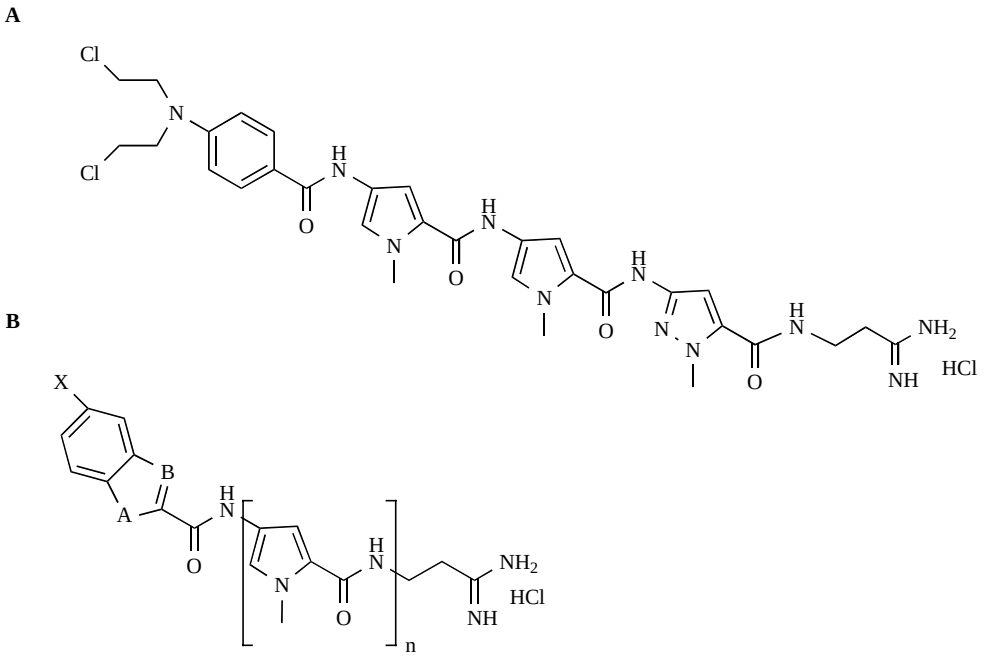
The group of Baraldi has described the synthesis and the structure–activity relationship of a series of benzoyl and cinnamoyl nitrogen mustards tethered to different benzo-heterocycles and to two N-methylpyrrole carboxamide units terminating with an amidine [25]. The obtained derivatives demonstrated a cytotoxic activity 2–50-fold lower as compared to that of tallimustine against human K562 leukemia cells. Particularly, the tallimustine isosters bearing an indole or a benzothiophene ring showed a 4-fold less cytotoxic effect than tallimustine; on the other hand the compounds with an N-methyl indole or benzofuran showed a reduction in cytotoxicity of 7- and 14-fold, respectively. The obtained results may suggest that these derivatives possibly prefer to bind to AT-rich sequences with a selectivity comparable to that of tallimustine [73].

### *C. Heterocyclic Distamycins*

Taken in mind that distamycins with a pyrrole 2-carbonyl moiety bearing alkylating groups, such as a BAM or an α-bromo acryloyl moieties, are characterized by high cytotoxicity on several tumor cell lines and *in vivo* antitumor activity; new potential minor groove binders as novel carriers to deliver DNA-reacting groups were proposed by Baraldi et al. [25].

The obtained compounds showed in general a cytotoxic activity much greater than that of the parent distamycin A [25]. It has to be underlined that the type of alkylating group had a great effect on the cytotoxicity of compounds showing the same oligopeptidic frame, indeed the compounds bearing an α-bromoacryloyl moiety were at least 60-fold more potent than the benzoyl mustard counterpart. In general, for tetra-oligopeptides owing the same alkylating moiety and a different N-terminal heterocycle, it was found that the pyrrole nucleus conferred an antiproliferative activity higher with respect to compound presenting the pyrazole and imidazole rings [74].

In order to obtain potentially more stable minor groove binders, aimed to improve the relative instability of the polypyrrolic skeleton, Baraldi et al. have reported the design, synthesis, *in vivo* and *in vitro* antileukemic activity of a series of distamycins with general formula reported in Fig. (**7B**). These derivatives are characterized by the presence of




X = 4-[$(ClCH_2CH_2)_2N$]$C_6H_4CONH$, $(ClCH_2CH_2)_2N$, $CH_2$=$CBrCONH$
A = NH, NMe, O, S
B = CH or N
n = 2 or 4

**Fig. (7).** Chemical structure of PNU157977 (**A**) and general formula of benzo-heterocyclic distamycins (**B**).

different benzoheterocyclic rings (i.e. indole, N-methyl indole, benzoimidazole, benzofuran) bearing a nitrogen mustard, or a benzoyl nitrogen mustard or an α-bromoacryloyl group as alkylating moieties, tethered to the distamycin frame. It was found that, the activities of derivatives bearing the same alkylating moiety are slightly affected by the type of the heteroatom within the benzoheterocyclic ring [74].

The obtained compounds showed in general an *in vitro* activity against L1210 murine leukemia cell line comparable to that of tallimustine. Moreover, the compounds having the nitrogen mustard and the α-bromoacryloyl moieties directly linked to benzoheterocyclic ring, displayed higher cytotoxic activities with respect to benzoyl nitrogen mustard derivatives of benzoheterocycles.

Among these compounds, the 5-nitrogen mustard N-methylindole derivative of distamycin showed the best antileukemic activity *in vivo* and was selected for further extensive evaluation.

The positive role of some modifications of the amidino moiety is also corroborated considering the α-bromoacryloyl pyrazole, imidazole, and benzoheterocyclic derivatives of distamycin A. In fact, some of these derivatives maintain or improve the antiproliferative activity of the parent compounds against mouse leukemia L1210 cells; whereas the imidazolic amidine derivatives showed a decreased activity in comparison to the parent compound, probably related to the different ability of the obtained molecule to enter the cell [75].

## 2. Use of Macromolecular Carriers

The relatively low therapeutic index of the clinically used alkylating agents is probably related to the unspecific DNA damage involving G-C rich stretches of DNA, thus inducing unselective growth inhibition and death both in neoplastic and in normal tissues.

A variety of approaches have been pursued to overcome these limitations, including (as previously reported) the synthesis of distamycin analogues and the use of specialized delivery systems. The use of delivery systems could be indeed useful as method to efficiently and selectively target antitumor agents to the tumor site, thus leading advantages in the clinical use of these potent drugs [76-78].

Concerning distamycins, only few papers are present in the literature proposing the use of delivery systems [79-81]. Particularly, among the known delivery systems, liposomes, ethosomes and micelles (Fig. **8**) have been considered as macromolecular carriers for distamycins [79-81].

Liposomes and ethosomes are known to enhance drug cellular internalization, to generally decrease unwanted systemic toxic effects, and increase the solubility of lipophilic drugs in biological fluids, while at the same time modulating the drug-release profile [82,83].

Furthermore, different studies have demonstrated that engineered phospholipid vesicles could achieve specific drug release to target cells by mean of passive or active targeting strategies [84-90].

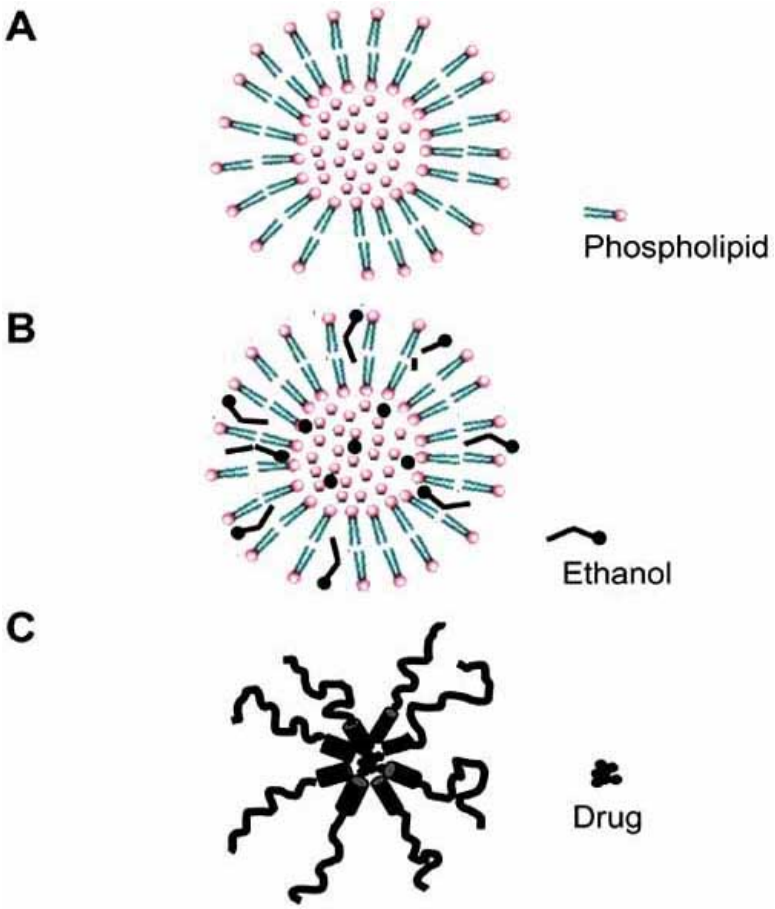



**Fig. (8).** Schematic representation of liposomes (**A**), ethosomes (**B**) and micellar systems (**C**).

In addition to liposomes, other formulations based on association colloids, such as micellar systems, have been proposed as efficient strategies for the administration of drugs with limited solubility in biological fluids [91-92].

With respect to micellar systems, the advantages offered can be outlined as (a) the increase of solubilization capacity of lipophilic compounds, (b) the modulation of both the pharmacokinetic and the bioavailability of the drug and (c) the stability and simplicity of preparation of micelles [86-89].

Moreover, the amphiphilic properties of the surfactants used to prepare micellar systems should potentially increase the permeability of the drug through biological membranes, which generally results in an enhanced intracellular drug concentration [93]. In this view, micellar systems could possibly contribute to an increase in the gastrointestinal absorption of distamycins, thus facilitating their oral administration [94].

### *A. Liposomes*

Liposomes (Fig. **8A**) are simple microscopic vesicles in which lipid bilayer structures are present with an aqueous volume entirely enclosed by a lipid membrane with phospholipid and cholesterol being the main ingredients. Phospholipids include phosphoglycerides and sphingolipids together with their hydrolysis products [95]. Cholesterol may be included to improve bilayers characteristics of liposomes [96]; increasing microviscosity of the bilayers, reducing permeability of the membrane to water soluble molecules, stabilizing the membrane and increasing rigidity of the vesicles.

Many methods for preparation of liposomes are described in the literature [96-98]. Most commonly, the film hydration method is used [99-100].

Liposomal drug delivery system is advantageous in the fulfillment of the aspects related to protection of the drug, controlled release of the active moiety along with the targeted delivery, and cellular uptake via endocytosis [101-104]. Besides the merits, liposomes also pose certain problems associated with degradation by hydrolysis [105], oxidation [106], sedimentation, drug leaching, aggregation or fusion [107] during storage. Approaches that can be used to increase liposome stability involve efficient formulation and freeze-drying.

As above reported, only few papers concerning liposome encapsulation of distamycins have been published. In particular, our group in 2004 studied the production and characterization of liposomes containing distamycin A and one of its alkyl derivatives (C16-Dist) [77]. Egg-phosphatidylcholine/cholesterol liposomes (4:1 mol/mol), prepared by reverse phase evaporation technique and extrusion, lead to the obtaining of vesicles with an encapsulation efficiency around 19.0% for distamycin A and almost complete for C16-Dist (99.8%). The *in vitro* antiproliferative activity of the distamycins-containing liposomes, determined on human leukaemic K562 cells, showed an increase of 11-fold and 8-fold for distamycin A and its alkyl derivative, respectively, as compared to that of the corresponding free drug. Taken together these results suggested that liposomal formulations could increase the activity and specificity of distamycins in experimental *in vitro* antitumor therapy.

Another paper of Cortesi *et al.* [79] studied the comparison of liposomes and micellar systems for the delivery and possible administration of distamycins.

It is well known that micellar solutions are able to influence the solubility and stability of lipophilic compounds in water. For example, lipophilic drugs can be solubilized by the hydrophobic environment within the micelles (direct micelles), allowing for improvements in the level of bioavailability [108].

Particularly, in the mentioned paper all the formulations were designed in order to increase the solubility of distamycins in aqueous environment and to reduce the possible toxicity problems related to the administration of these drugs. For instance, liposomes were prepared by reverse phase evaporation technique and extrusion, then characterized in term of dimensions, morphology and encapsulation efficacy. The analysis of their *in vitro* antiproliferative activity on cultured human and mouse leukemic cells demonstrated that liposomes and micellar solutions containing distamycins exert quite different effects as compared to that shown by the free drug depending on the type of drug and also of the cell line used. Our paper considered the influence of the delivery system on the activity of a wide number of distamycins characterized by different physico-chemical properties (e.g. molecular weight, solubility, interaction with phospholipid, presence of charged groups, hydrophilic/lipophilic balance…) [79-81]. These chemical modifications were introduced in the attempt to obtain more stable compounds, possibly increasing the binding to DNA and selectivity of alkylation with a concomitant reduction of the adverse effects characterizing the pharmacological profile of distamycin A [21].

In particular, the activity of the natural distamycin A (reference compound) and several semi-synthetic analogues, that more or less strictly resemble the molecular shape of the reference compound, were considered [21].

It was demonstrated that, the activity of distamycins released by specialized delivery systems is in many cases higher with respect to the correspondent drug tested in the free form. It should be underlined that as control, the antiproliferative effects of the empty delivery systems on both cell lines were evaluated. The obtained results demonstrated that vehicles do not cause any inhibition of cell growth, thus suggesting that the enhanced effect of distamycins (where it happens) could be reasonably due to the increased solubility and/or bioavailability of the encapsulated compounds [80].

### *B. Ethosomes*

Ethosomes (Fig. **8B**) are lipid carriers developed in this decade by Touitou *et al.* [83,109-110]. The ethosomal system is composed of phospholipid, ethanol and water [110]. Ethosomes are most commonly prepared as follows. The lipids and the drug are dissolved in ethanol; then the aqueous solution is slowly added in a fine stream at constant rate in a well-sealed container with constant mixing. Several studies investigated the effect of ethanol on physicochemical characteristics of the ethosomal vesicles [109-114]. One

reported characteristic of ethosomes is their small size relative to liposomes, due to the incorporation of ethanol [110-112]. Precisely, the higher the concentration (in the range of 20–45%), the lower the size of ethosomal vesicles [109]. Ethosomes exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles [109] as well as by the presence of ethanol in ethosomes, which allows for better solubility of many drugs.

In a recent study of Cortesi *et al.* [81], a comparative study of the performances of liposomes and ethosomes as specialized delivery systems for distamycin A and two its benzo-heterocyclic derivatives was described. Liposomes and ethosomes were prepared by classical methods extruded through polycarbonate filters and then characterized in term of size, morphology and encapsulation efficiency. The percentage of association of distamycin A was the same both in liposomes and in ethosomes (around 16.0%), while the two distamycin derivatives showed different results depending on the types of vesicles. Particularly, the association yield was around 80% in liposomes and around 50% in ethosomes. This difference was probably due to the presence of embedded ethanol within ethosomes that influence the hydrophobic environment of the vesicle bilayer. In fact, it was supposed that due to their lipophilic characteristics, distamycins are reasonably expected to be associated (intercalated) within the vesicle phospholipid bilayer. However, from the analysis of the freeze-fracture photographs, no appreciable alteration of the bilayer structure, due to a possible interaction with the drug, was evident. The analysis of the *in vitro* antiproliferative activity of distamycin A and its benzoheterocyclic derivatives on cultured human K562 and mouse leukemic L1210 cells demonstrated that the drugs administrated within vesicles are more effective than the corresponding free form used in the same conditions.

## CONCLUSIONS

This review reported the main advances of the knowledge concerning distamycins. Particularly these compounds showed interesting cytotoxicity both *in vitro* and *in vivo* as anticancer molecules representing a possible promise of clinical efficacy.

With the objective to identify promising candidates, distamycins have been used as DNA minor groove sequence-selective vector of alkylating moieties, in which the formyl group has been substituted by benzoyl nitrogen mustard (BAM), *para*-phenylbutanoic nitrogen mustard (chlorambucil, CHL), cinnamoyl nitrogen mustard, halogenoacryloyl, *O*-methyl sulfonate ester, and epoxycarbonyl moieties, disclosing the possibility of obtaining compounds endowed with relevant cytotoxic and antitumor activity than distamycin A [43-44].

Hybrid compounds, in which known antitumor compounds or simple active moieties of known antitumor agents have been tethered to distamycin frame, have been extensively reviewed in the recent past [115]. The nature of antitumor agents and therefore also the rationale that led to these compounds were different. The strategy is represented by hybrid molecules, combining derivatives of naturally occurring alkylating agent or moiety, with a distamycin-like minor groove binder, with the aim of combining high DNA affinity and sequence selectivity with chemically reactive functions. In general the interaction with DNA tends to be dominated by the minor groove-binding moiety, that is, the conjugates bind to the minor groove with preferential interaction with AT-rich sequences.

In consideration of their potential therapeutic properties, the synthesis of new distamycin derivatives and the development of controlled delivery strategies, could lead to significant advantage in the clinical use of these molecules possibly overcoming or mitigating the low solubility, specificity and toxicity problems associated with their use. The development of an efficient drug entrapment protocol and studies on *in vitro* activity are, in fact, essential prerequisites to any reproducible clinical trials. The encouraging results reported in the recent literature suggest that the enhancement of drug activity expressed by delivery systems-associated distamycins could be interesting in order to obtain an efficient therapeutic effect aimed to reduce or minimize the toxicity occurring with high dosage of distamycins.

Based on these results, the overview here reported could be an interesting starting point for future experimental therapy of distamycins.

## ACKNOWLEDGEMENTS

Authors thank Fondazione Cassa di Risparmio di Cento and Regione Emilia Romagna for financial support.

## REFERENCES

[1] Arcamone, F.; Penco, S.; Orezzi, P.G.; Nicolella, V.; Pirelli, A. Structure and synthesis of distamycin A. *Nature*, **1964**, *203*, 1064-65.

[2] Grehn, L.; Ragnarsson, U. Novel efficient total synthesis of antiviral antibiotic distamycin A. *J. Org. Chem.*, **1981**, *46*, 3492-97.

[3] Baird, E.E.; Dervan, P.B. Solid phase synthesis of polyamides containing imidazole and pyrrole amino acids. *J. Am. Chem. Soc.*, **1996**, *118*, 6141-46.

[4] Matteoli, B.; Bernardini, S.; Iuliano, R.; Parenti, S.; Freer, G.; Broccolo, F.; Baggiani, A.; Subissi, A.; Arcamone, F.; Ceccherini-Nelli, L. *In vitro* antiviral activity of distamycin A against clinical isolates of herpes simplex virus 1 and 2 from transplanted patients. *Intervirology*, **2008**, *51*, 166-72.

[5] Lee, S.; Inselburg, J. *In vitro* sensitivity of *Plasmodium falciparum* to drugs that bind DNA or inhibit its synthesis. *J. Parasitol.*, **1993**, *79*, 780-82.

[6] Pelton, J.G.; Wemmer, D.E. Binding modes of distamycin A with d(CGCAAATTTGCG)2 determined by two dimensional NMR. *J. Am. Chem. Soc.*, **1990**, *112*, 1393-99.

[7] Baraldi, P.G.; Bovero, A.; Fruttarolo, F.; Preti, D.; Tabrizi, M.A.; Pavani, M.G.; Romagnoli, R. DNA minor groove binders as potential antitumor and antimicrobial agents. *Med. Res. Rev.*, **2004**, *24*, 475-528.

[8] Baraldi, P.G.; Nunez, C.; Espinosa, A.; Romagnoli, R. Distamycin A as stem of DNA minor groove alkylating agents. *Curr. Top. Med. Chem.*, **2004**, *4*, 231-39.

[9] Sharma, S.; Doherty, K.M.; Brosh Jr., R.M. DNA helicases as targets for anti-cancer drugs. *Curr. Med. Chem. Anticancer Agents*, **2005**, *5*, 183-99.

[10] Beerman, T.A.; Woynarowski, J.M.; Sigmund, R.D.; Gawron, L.S.; Rao, K.E.; Lown, J.W. Netropsin and bis-netropsin analogs as inhibitors of the catalytic activity of mammalian DNA topoisomerase II and topoisomerase cleavable complexes. *Biochim. Biophys. Acta*, **1991**, *1090*, 52-60.

[11] McHugh, M.M.; Woynarowski, J.M.; Sigmund, R.D.; Beerman, T.A. Effect of minor groove binding drugs on mammalian topoisomerase I activity. *Biochem. Pharmacol.*, **1989**, *38*, 2323-28.

[12] Woynarowski, J.M.; McHugh, M.; Sigmund, R.D.; Beerman, T.A. Modulation of topoisomerase II catalytic activity by DNA minor groove binding agents distamycin, Hoechst 33258, and 4,6-diamidine-2-phenylindole. *Mol. Pharmacol*., **1989**, *35*, 177-82.

[13] Mote Jr., J.; Ghanouni, P.; Reines, D. A DNA minor groove binding ligand both potentiates and arrests transcription by RNA polymerase II. *J. Mol. Biol.* **1994**, *236*, 725-37.

[14] Sutherland, G.R.; Jacky, P.B.; Baker, E.G. Heritable fragile sites on human chromosomes. XI. Factors affecting expression of fragile sites at 10q25, 16q22, and 17p12. *Am. J. Hum. Genet.*, **1984**, *36*,110-22.

[15] Hsu, Y.Y.; Wang, Y.H. Human fragile site FRA16B DNA excludes nucleosomes in the presence of distamycin. *J. Biol. Chem.* **2002**, *277*, 17315-19.

[16] Cozzi, P. Recent outcome in the field of distamycin-derived minor groove binders. *Farmaco*, **2000**, *55*, 168-73.

[17] Dervan, P.B.; Doss, R.M.; Marques, M.A. Programmable DNA binding oligomers for control of transcription. *Curr. Med. Chem. Anticancer Agents,* **2005**, *5*, 373-87.

[18] Dervan, P.B.; Edelson, B.S. Recognition of the DNA minor groove by pyrrole-imidazole polyamides. *Curr. Opin. Struct. Biol.*, **2003**, *13*, 284-90.

[19] S. Neidle, DNA minor-groove recognition by small molecules. *Nat. Prod. Rep*., **2001**, *18*, 291-309.

[20] Wemmer, D.E. Designed sequence- specific minor groove ligands. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 439-461.

[21] Wang, H.; Laughton, C.A. Molecular modelling methods for prediction of sequence- selectivity in DNA recognition. *Methods*, **2007**, *42*, 196-203.

[22] Sabatino, M.A.; Colombo, T.; Geroni, C.; Marchini, S.; Broggini, M. Enhancement of *in vivo* antitumor activity of classical anticancer agents by combination with the new, glutathione interacting DNA minor groove-binder, brostallicin. *Clin. Cancer Res*., **2003**, 9, 5402-08.

[23] Goodsell, D.S. Sequence recognition of DNA by lexitropsins. *Curr. Med. Chem.* **2001**, *8*, 509-16.

[24] Baraldi, P.G.; Romagnoli, R.; Beria, I.; Cozzi, P.; Geroni, C.; Mongelli, N.; Bianchi, N.; Mischiati, C.; Gambari, R. Synthesis and antitumor activity of new benzoheterocyclic derivatives of distamycin A. *J. Med. Chem.*, **2000**, *43*, 2675-84.

[25] Agen, C.; Sironi, A.M.; Danesi, R.; Costa, M.; Bernardini, N.; Paparelli, A.; Della Torre, P.; Del Tacca, M. Characterization of the toxicity of distamycin derivatives on cancer cell lines and rat heart. *Toxicology*, **1992**, *75*, 209-19.

[26] Lian, T.; Ho, R.J.Y. Trends and developments in liposome drug delivery systems. *J. Pharm. Sci.*, **2001**, *90*, 667-80.

[27] Khedkar, S.A.; Malde, A.K.; Coutinho, E.C. CoMFA study of distamycin analogs binding to the minor-groove of DNA: a unified model for broad-spectrum activity. *J. Mol. Model.*, **2007**, *13*,1099-1108.

[28] Woods, C.R.; Faucher, N.: Eeschgfaller, B.: Bair, K.W.; Boger, D. Synthesis and DNA binding properties of saturated distamycin analogues. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 2647-50.

[29] Bhattacharya, S.; Thomas, M. DNA binding properties of novel distamycin analogs that lack the leading amide unit at the N-terminus. *Biochem. Biophys. Res. Commun*., **2000**, *267*, 139-44.

[30] Bhattacharya, S.; Thomas, M. Facile synthesis of oligopeptide distamycin analogs devoid of hydrogen bond donors or acceptors at the N-terminus: Sequence-specific duplex DNA binding as a function of peptide chain length. *Tetrahedron Lett.*, **2000**, *41*, 5571-75.

[31] Zaffaroni, N.; Lualdi, S.; Villa, R.; Bellarosa, D.; Cermele, C.; Felicetti, P.; Rossi, C.; Orlandi, L.; Daidone, M.G. Inhibition of telomerase activity by a distamycin derivative: Effects on cell proliferation and induction of apoptosis in human cancer cells. *Eur. J. Cancer,* **2002**, *38*, 1792-801.

[32] Fukuda, Y.; Seto, S.; Furuta, H.; Ebisu, H.; Oomori, Y.; Terashima, S. The novel cyclopropapyrroloindole (CPI) bisalkylators bearing 3,3 0-(1,4-phenylene)diacryloyl group as a linker. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 2003-04.

[33] Dyatkina, N.B.; Roberts, C.D.; Keicher, J.D.; Dai, Y.; Nadherny, J.P.; Zhang, W.; Schmitz, U.; Kongpachith, A.; Fung, K.; Novikov, A.A.; Lou, L.; Velligan, M.; Khorlin, A.A.; Chen, M.S. Minor groove DNA binders as antimicrobial agents. 1. Pyrrole tetramides are potent antibacterial against Vancomycin resistant Enterococci and Methicillin resistant Staphylococcus aureus. *J. Med. Chem*., **2002**, *45*, 805-17.

[34] Wang, W.; Lown, J.W. Anti-HIV-1 activity of linked lexitropsins. *J. Med. Chem.*, **1992**, *35*, 2890-97.

[35] Neamati, N.; Mazumder, A.; Sunder, S.; Owen, J.M.; Tandon, M.; Lown, J.W., Pommier, Y. Highly potent synthetic polyamides, bisdistamycins and lexitropsins as inhibitors of human immunodeficiency virus type 1 integrase. *Mol. Pharm.*, **1998**, *54*, 280-90.

[36] Howard, O.M.Z.; Oppenheim, J.J.; Hollingshead, M.G.; Covey, J.M.; Bigelow, J.; Mc Cormack, J.J.; Buckheit, R.W.; Clanton, D.J.; Turpin, J.A.; Rice, W.G. Inhibition of *in vitro* and *in vivo* HIV replication by a dystamycin analogue that interferes with chemochine receptor function: a candidate for chemotherapeutic and microbicidal application. *J. Med. Chem*., **1998**, *41*, 2184-93.

[37] Ryabinin, V.A.; Zakharova, O.D.; Yurchenko, E.Y.; Timofeeva, O.A.; Martyanov, IV, Tokarev, A.A.; Belanov, E.F.; Bormotov, N.I.; Tarrago-Litvak, L.; Andreola, M.L.; Litvak, S.; Nevinsky, G.A.; Sinyakov, A.N. Synthesis and evaluation of oligo-1,3-thiazolecarboxamide derivatives as HIV-1 reverse transcriptase inhibitors. *Bioorg. Med. Chem*., **2000**, *8*, 985-93.

[38] Burli, R.W.; Ge, Y.; White, S.; Baird, E.E.; Touami, S.M.; Taylor, M.; Kaizerman, J.A.; Moser, H.E. DNA binding ligands with excellent antibiotic potency against drug-resistant gram-positive bacteria. Bioorg. *Med. Chem. Lett.*, **2002**, *12*, 2591-94.

[39] Arcamone, F.M.; Animati, F.; Barbieri, B.; Configliacchi, E.; D'Alessio, R.; Geroni, C.; Giuliani, F.C.; Lazzari, E.; Menozzi, M.; Mongelli, N.; Penco, S.; Verini.; M.A. Synthesis, DNA-binding properties, and antitumor activity of novel distamycin derivatives. *J. Med. Chem*., **1989**, *32*, 774-78.

[40] D'Alessio, R.; Geroni, C.; Biasioli, G.; Pesenti, E.; Grandi, M.; Mongelli, N. Structure–activity relationship of novel distamycin A derivatives: Synthesis and antitumor activity. *Bioorg. Med. Chem. Lett.*, **1994**, *4*, 1467-72.

[41] Pezzoni, G.; Grandi, M.; Biasioli, G.; Capolongo, L.; Ballinari, D.; Giuliani, F.C.; Barbieri, B.; Pastori, A.; Pesenti, E.; Mongelli, N.; Spreafico, F. Biological profile of FCE 24517, a novel benzoyl mustard analog of distyamycin A. *Br. J. Cancer*, **1991**, *64*, 1047-50.

[42] Marchini, S.; Cirò, M.; Gallinari, F.; Geroni, C.; Cozzi, P.; D'Incalci, M.; Broggini, M. α-bromoacryloyl derivative of distamycin A (PNU 151807): A new non covalent minor groove binder with antineoplastic activity. *Br. J. Cancer*, **1999**, *80*, 991-97.

[43] Cozzi, P.; Beria, I.; Caldarelli, M.; Capolongo, L.; Geroni, C.; Mongelli, N. Cytotoxic halogenoacrylic derivatives of distamycin A. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1269-72.

[44] Cozzi, P.; Beria, I.; Caldarelli, M.; Geroni, C.; Mongelli, N.; Pannella, G. Cyotoxic α-bromoacrylic derivatives of distamycin analogues modified at the amidino moiety. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1273-76.

[45] Geroni, C.; Pannella, G.; Capolongo, L. Antitumor activity of PNU-166196, a novel DNA minor groove binder selected for clinical development. *Proc. Am. Assoc. Cancer Res.*, **2000**, *41*, 265-67.

[46] Pezzoni, G.; Grandi, M.; Biasoli, G.; Capolongo, L.; Ballinari, D.; Giuliani, F.C.; Barbieri, B.; Pastori, A.; Pesenti, E.; Mongelli, N.; Spreafico, F. Biological profile of FCE 24517, a novel benzoyl mustard analog of distamycin A. *Br. J. Cancer*, **1991**, *64*, 1047-50.

[47] Bielawski, K.; Bielawska, A. Small-molecule based delivery systems for alkylating antineoplastic compounds. *ChemMedChem*, **2008**, *3*, 536-42.

[48] Ghielmini, M.; Bosshard, G.; Capolongo, L.; Geroni, C.; Pesenti, E.; Torri, V.; D'Incalci, M.; Cavalli, F.; Sessa, C. Estimation of the haematological toxicity of minor groove alkylators using tests on human cord blood cells. *Br. J. Cancer*, **1997**, *75*, 878-83.

[49] Viallet, J.; Stewart, D.; Shepherd, F.; Ayoub, J.; Cormier, Y.; Di Pietro, N.; Steward, W. Tallimustine is inactive in patients with previously treated small-cell lung cancer—A phase II trial of the National Cancer Institute of Canada clinical trials group. *Lung Cancer*, **1996**, *15*, 367-73.

[50] Sessa, C.; Pagani, O.; Zurlo, M.G.; de Jong, J.; Hofmann, C.; Lassus, M.; Marrari, P.; Strolin Benedetti, M.; Cavalli, F. Phase I study of the novel distamycin derivative tallimustine (FCE 24517). *Ann. Oncol*., **1994**, *5*, 901-07.

[51] Bigoni, M.; Salvatore, C.; Palma, C.; Manzini, S.; Animati, F.; Lombardi, P.; Pratesi, G.; Supino, R.; Zunino, F. Cytotoxic and antitumor activity of MEN 10710, a novel alkylating derivative of distamycin. *Anti-Cancer Drugs*, **1997**, *8*, 845-52.

[52] Bartulewicz, D.; Bielawski, K.; Bielawska, A.; Rozanski, A. Synthesis, molecular modelling, and antiproliferative and cytotoxic effects of carbocyclic derivatives of distamycin with chlorambucil moiety. *Eur. J. Med. Chem*. **2001**, *36*, 461-67.

[53] Bardos, T.J.; Datta-Gupta, N.; Hebborn, P.; Triggle, D.J. A study of comparative chemical and biological activities of alkylating agents. *J. Med. Chem.* **1964**, *8*, 167-74.

[54] Schmid, F.A.; Otter, G.M.; Stock, C.C. Resistance patterns of walker carcinosarcoma 256 and other rodent tumors to cyclophosphamide and L-phenylalanine mustard. *Cancer Res.*, **1990**, *40*, 830-33.

[55] Cozzi, P.; Beria, I.; Biasoli, G.; Caldarelli, M.; Capolongo, L.; D'Alessio, R.; Geroni, C.; Mazzini, S.; Ragg, E.; Rossi, C.; Mongelli N. Novel phenyl nitrogen mustard and half-mustard derivatives of distamycin A. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 2985-90.

[56] Baraldi, P.G.; Beria, I.; Cozzi, P.; Geroni, C.; Espinosa, A.; Gallo, M.A.; Entrena, A.; Bingham, J.P.; Hartley J.A.; Romagnoli R. Cinnamoyl nitrogen mustard derivatives of pyrazole analogues of tallimustine modified at the amidino moiety: design, synthesis, molecular modeling and antitumor activity studies. *Bioorg. Med. Chem*., **2004**, *14*, 3911-21.

[57] Cozzi, P.; Beria, I.; Caldarelli, M.; Capolongo, M.; Geroni, C.; Mazzini, S.; Ragg, E. Phenyl sulfur mustard derivatives of distamycin A. *Bioorg. Med. Chem. Lett*., **2000**, *10*, 1653-56.

[58] Lee, M.; Simpson, J.E.; Woo, S.; Kaenzig, C.; Anlezark, G.M.; Eno-Amooquaye, E.; Burke, P.J. Synthesis of an aminopropyl analog of the experimental anticancer drug tallimustine and activation of its 4-nitrobenzylcarbamoyl prodrug by nitroreductase and NADH. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1065–70.

[59] Cozzi, P.; Beria, I.; Biasoli, G.; Caldarelli, M.; Capolongo, L.; Geroni, C.; Mongelli, N. Novel phenyl nitrogen mustard and half-mustard derivatives of amidino-modified distamycin A. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 2979-84.

[60] Wyatt, M.D.; Garbiras, B.J.; Haskell, M.K.; Lee, M.; Souhami, R.L.; Hatley, J.A. Structure–activity relationship of a series of nitrogen mustard- and pyrrole-containing minor groove-binding agents related to distamycin. *Anti-Cancer Drug Des.*, **1994**, *9*, 511-25.

[61] Baraldi, PG, Cozzi, P, Geroni, C, Mongelli, N, Romagnoli, R, Spalluto, G. Novel benzoyl nitrogen mustard derivatives of pyrazole analogues of distamycin A: Synthesis and antitumor activity. *Bioorg. Med. Chem.* **1999**, *17*, 251-62.

[62] Marchini, S.; Cozzi, P.; Beria, I.; Geroni, C.; Capolongo, L.; D'Incalci, M.; Broggini, M. Sequence-specific DNA alkylation of novel tallimustine derivatives. *Anti-Cancer Drug Des.*, 1998, 13, 193-205.

[63] Baraldi, P.G.; Beria, I.; Cacciari, B.; Capolongo, L.; Cozzi, P.; Mongelli, N.; Romagnoli, R.; Spalluto, G. Structure–activity relationship of novel tallimustine derivatives: Synthesis and antitumor activity. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 1247-52.

[64] Lee, H.H.; Boyd, M.; Gravatt, G.L.; Denny, W.A. Pyrazole analogues of the bispyrrolecarboxamide anti-tumor antibiotics: Synthesis, DNA binding and anti-tumor properties. *Anti-Cancer Drug Des.*, **1991**, *6*, 501-17.

[65] Cozzi, P. Distamycin derivatives as potential anticancer agents. *IDrugs*, **2001**, *4*, 573-81

[66] Baraldi, P.G.; Romagnoli, R.; Spalluto, G.; Cozzi, P.; Mongelli, N.; Bianchi, N.; Gambari, R. DNA sequence recognizing properties of minor groove alkylating agents: Effects of the replacement of N-methylpyrrole by an N-methylimidazole on tallimustine and its own homologous. *Arzneim. Forsh. Drug Res*., **2000**, *50*, 309-15.

[67] Lee, M.; Rhodes, A,L.; Wyatt, M.D.; D'Incalci, M.; Forrow, S.; Hartley, J.A. *In vitro* cytotoxicity of GC sequence directed alkylating agents related to distamycin. *J. Med. Chem.*, **1993**, *36*, 863-70.

[68] Hartley, J.A.; Wyatt, M.D.; Garbiras, B.J.; Richter, C.B.J.; Richter, C.; Lee, M. Probing the importance of the second chloroethyl arm of a benzoic acid mustard derivative of an imidazole-containing analogue of distamycin. *Bioorg. Med. Chem. Lett*., **1994**, *4*, 2421-24.

[69] Xie, G.; Gupta, R.; Lown, J.W. Design, synthesis, DNA sequence preferential alkylation and biological evaluation of N-mustard derivatives of distamycin and netropsin analogues. *Anti-Cancer Drug Des.*, **1995**, *10*, 389-409.

[70] Romagnoli, R.; Baraldi, P.G.; Iaconinoto, M.A.; Carrion, M.D.; Tabrizi, M.A.; Gambari, R.; Borgatti, M.; Heilmann, J. Synthesis and biological activity of alpha-bromoacryloyl lexitropsin conjugates. *Eur. J. Med. Chem.*, **2005**, *40*, 1123-28.

[71] Baraldi, P.G.; Beria, I.; Cacciari, B.; Capolongo, L.; Cozzi, P.; Franzetti, C.; Mongelli, N.; Romagnoli, R, Spalluto, G. Synthesis and antitumor activity of novel distamycin derivatives. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 1241-46.

[72] Baraldi, P.G.; Balboni, G.; Romagnoli, R.; Spalluto, G.; Cozzi, P.; Geroni, C.; Mongelli, N.; Rutigliano, C.; Bianchi, N.; Gambari, R. PNU 157977: A new potent antitumor agent exhibiting low *in vivo* toxicity in mice injected with L1210 leukemia cells. *Anti-Cancer Drug Des.*, **1999**, *14*, 71-76.

[73] Baraldi, P.G.; Romagnoli, R.; Pavani, M.G.; Nuñez, M.D.C.; Bingham, J.P.; Hartley, J.A. Benzoyl and cinnamoyl nitrogen mustard derivatives of tallimustine benzoetherocyclic analogues: Synthesis and antitumor activity. *Bioorg. Med. Chem.*, **2002**, *10*, 1611-18.

[74] Baraldi, P.G.; Romagnoli, R.; Beria, I.; Cozzi, P.; Geroni, C.; Mongelli, N.; Bianchi, N.; Mischiati, C.; Gambari, R. Synthesis and antitumor activity of new benzoheterocyclic derivatives of distamycin A. *J. Med. Chem.*, **2000**, *43*, 2675-84.

[75] Baraldi, P.G.; Beria, I.; Cozzi, P.; Bianchi, N.; Gambari, R.; Romagnoli, R. Synthesis and growth inhibition activity of α-bromoacrylic heterocyclic and benzoheterocyclic derivatives of distamycin A modified on the amidino moiety. *Bioorg. Med. Chem*., **2003**, *11*, 965-975.

[76] Briones, E.; Colino, C.I.; Lanao, J.M. Delivery systems to increase the selectivity of antibiotics in phagocytic cells. *J. Control. Release*, **2008**, *125*, 210-227.

[77] Kshirsagar, N.A. Drug delivery systems. *Indian J. Pharmacol*., **2000,** *32*, S54-S61.

[78] Misra, A.; Ganesh, S.; Shahiwala, A.; Shah, S.P. Drug delivery to the central nervous system: a review. *J. Pharm. Pharm. Sci*., **2003**, *6*, 252-73.

[79] Cortesi, R.; Esposito, E.; Cuccu, I.; Zaid, A.N.; Romagnoli, R.; Menegatti, E.; Nastruzzi, C. Liposomes and micellar solutions for the delivery of some benzoheterocyclic derivatives of distamycin A. *Drug Deliv*., **2007**, *14*, 1-8.

[80] Cortesi, R.; Romagnoli, R.; Cervellati, F.; Menegatti, E.; Esposito, E.; Nastruzzi, C. Liposome containing distamycins: preparation, characterization and antiproliferative activity. *Drug Deliv*., **2004**, *11*, 1-6.

[81] Cortesi, R.; Zaid, A.N.; Romagnoli, R.; Menegatti, E.; Esposito, E. Liposomes and ethosomes for the delivery of distamycin A derivatives': a comparative study. *J. Liposome Res*. In press.

[82] Sharma, A., Sharma, U.S. Liposomes in drug delivery: Progress and limitations. *Int. J. Pharm.*, **1997**, *154*, 123-140.

[83] Touitou, E.; Alkabes, M.; Dayan, N. Ethosomes: novel lipid vesicular system for enhanced delivery. *Pharm. Res.*, **1997**, *S14*, 305-06.

[84] Cortesi, R.; Esposito, E.; Maietti, A.; Menegatti, E., Nastruzzi, C. Formulation study for the antitumor drug camptothecin: liposomes, micellar solutions and microemulsions. *Int. J. Pharm.* **1997**,*159*, 95-103.

[85] Gabizon, A. In *Drug Carrier System*; Roerdink, F.H.D. and Kroo, A.M., Eds.; John Wiley & Sons Ltd., Chichester, UK, **1989**, pp.185-211.

[86] Chonn, A.; Cullis, P.R. Recent advances in liposomal drug-delivery systems. *Curr. Opin. Biotechnol.*, **1995**, *6*, 698-708.

[87] Pavliv, L.; Tyle, P.; Johnston, A. Liposomal formulations of a novel antitumor agent: drug loading and optimization. *PDA J. Pharm. Sci. Technol.*, 1995, *49*, 9-12.

[88] Mori, A.; Kennel, S.J.; Huang, L. Immunotargeting of liposomes containing lipophilic antitumor prodrugs. *Pharm. Res.*, **1993**, *10*, 507-514.

[89] Griffin, T.; Rybak, M.E.; Recht, L.; Singh, M.; Salimi, A.; Raso, V. Potentiation of antitumor immunotoxins by liposomal monesin, *J. Natl. Cancer. Inst.*, **1993**, 85, 292-98.

[90] Sugarman, S.M.; Perez-Soler, R. Liposomes in the treatment of malignancy: A clinical perspective. *Crit. Rev. Oncol. Hematol.*, **1992**, *12*, 231-42.

[91] De Oliveira, A.G., Chaimovich, H. Effect of detergents and other amphiphiles on the stability of pharmaceutical drugs *J. Pharm. Pharmacol.*, **1993**, *45*, 850-861.

[92] Attwood, D. In *Colloidal Drug Delivery Systems*. Kreuter J., Ed.; Marcel Dekker, Inc, New York, USA., **1994**, pp. 31-71.

[93] Kakemi, K.; Sezaki, H.; Konishi, R.; Kimura, T.; Murakami, H. Effect of bile salts on the gastrointestinal absorption of drugs. I. *Chem. Pharm. Bull.*, **1990**, *18*, 275-80.

[94] Dancey, J.; Eisenhauer, E.A. Current perspectives on camptothecins in cancer treatment. *Br. J. Cancer*, **1996**, *74*, 327-338.

[95] Eibl, H. Synthesis of glycerophospholipids. *Chem. Phys. Lipids*, **1980**, *26*, 405-29.

[96] Vemuri, S.; Rhodes, C.T. Preparation and characterization of liposomes as therapeutic delivery systems: a review. *Pharm. Acta Helv*., **1995**, *70*, 95-111.

[97] Barenholz, Y.; Crommelin, D.J.A. In *Encyclopedia of Pharmaceutical Technology*; Swarbrick, J. and Boylan, J.C., Eds.; Marcel Dekker Inc.: New York, **1994**, *Vol. 9*, pp. 1–39.

[98] Weiner, N.; Martin, F.; Riaz, M. Liposomes as a drug delivery system. *Drug Dev. Ind. Pharm.*, **1989**, *15*, 1523-54.

[99] Williams, A. *Transdermal and Topical Drug Delivery, From theory to clinical practice*, 1st ed, Pharmaceutical Press: London **2003**.

[100] Biju, S.S.; Talegaonkar, S.; Mishra, P.R.; Khar, R.K. Vesicular Systems: an overview. *J. Pharm. Sci.*, **2006**, *68*, 141-153.

[101] Allen, T.M.; Hansen, C.B.; Stuart, D.D. In *Medical Applications of Liposomes;* Lasic, D.D. and Papahadjopoulos, D. Eds.; Elsevier: Amsterdam, **1998**, pp. 297-312.

[102] Allen, T.M.; Ahmed, I.; Lopes de Menezes, D.E.; Moase, E.H. *Biochem. Soc. Trans.*, **1995**, *23*, 1073-75.

[103] Kirpotin, D.B. In *Medical Applications of Liposomes;* Lasic, D.D. and Papahadjopoulos, D. Eds.; Elsevier: Amsterdam, **1998**, pp. 325-338.

[104] Mayer, L.D.; Cullis, P.R.; Bally, M.B. In *Medical Applications of Liposomes;* Lasic, D.D. and Papahadjopoulos, D. Eds.; Elsevier: Amsterdam, **1998**, pp. 231-249.

[105] Frfkjaer, S.; Hjorth, E.; Wfatis, O. In *Liposome Technology*; Gregoriadis, G. Ed; CRC Press: Boca Raton, FL, **1984**, *Vol 1*, 235-246.

[106] Hunt, C.; Tsang, S. α-Tocopherol retards autoxidation and prolongs the shelf-life of liposomes *Int. J. Pharm.*, **1981**, *8*, 101-110.

[107] Wong, M.; Thompson, T. Aggregation of dipalmitoylphosphatidyl-choline vesicles. *Biochemistry*, **1982**, *21*, 4133-39.

[108] Cortesi, R.; Nastruzzi, C. Liposomes, micelles and microemulsions as new delivery systems for cytotoxic alkaloids. *Pharm. Sci. Technol. Today*, **1999**, *2*, 288-98.

[109] Touitou, E.; Dayan, N.; Bergelson, L.; Godin, B.; Eliaz, M. Ethosomes - novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J. Control. Release* **2000**, *65*, 403-18.

[110] Touitou, E.; Godin, B.; Weiss, C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Dev. Res.*, **2000**, *50*, 406-15.

[111] Dayan, N.; Touitou, E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. *Biomaterials*, **2000**, *21*, 1879-85.

[112] Lopez-Pinto, J.M.; Gonzalez-Rodriguez, M.L.; Rabasco, A.M. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. *Int. J. Pharm.*, **2005**, *298*, 1-12.

[113] Elsayed, M.M.; Abdallah, O.Y.; Naggar, V.F.; Khalafallah, N.M. Deformable liposomes and ethosomes as carriers for skin delivery of ketotifen. *Pharmazie*, **2007**, *62*, 133-37.

[114] Esposito, E.; Menegatti, E.; Cortesi, R. Ethosomes and liposomes as topical vehicles for azelaic acid: a preformulation study. *J. Cosmet. Sci.*, **2004**, *55*, 253-264.

[115] Baraldi, P.G.; Preti, D.; Fruttarolo, F.; Tabrizi, M.A.; Romagnoli, R. Hybrid molecules between distamycin A and active moieties of antitumor agents. *Bioorg. Med. Chem.*, **2007**, 15, 17-35.

Received: May 19, 2009 Revised: December 11, 2009 Accepted: January 20, 2010