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Review

DNA minor-groove binders: results and design of new antitumor agents☆

Pier Giovanni Baraldi *, Barbara Cacciari, Andrea Guiotto, Romeo Romagnoli, Abdel Naser Zaid, Giampiero Spalluto

Dipartimento di Scienze Farmaceutiche, Universitá di Ferrara, Via Fossato di Mortara 17–19, I-44100 Ferrara, Italy

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Abstract

DNA minor-groove binding drugs have been extensively studied in the last years in order to influence the regulation of gene expression in neoplastic disorders by means of specific interactions with DNA bases. Pyrrolo[2,1-c][1,4]benzodiazepines (PBDs), CC-1065 and distamycins are three classes of minor-groove alkylating agents which showed interesting cytotoxicity profiles, but they cannot be used in humans for various toxicity problems. For this reason many groups applied heterocyclic substitutions extensively, in order to either modify the reactivity profile or introduce extra interactions within the minor groove, thus changing the binding site or modulating the binding sequence.

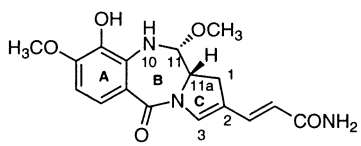
Keywords: Antitumor agents; CC-1065; Distamycin; PBD

1. Introduction

In recent years, many efforts have been focused at targeting specific DNA sequences with synthetic ligands, with the aim at designing both leads for medicinal chemistry and molecular probes for DNA polymorphism. In particular, DNA minor-groove binders represent a class of agents with several biological activities. The DNA-binding properties of these agents have all been extensively examined. These studies provided insights into the structural and functional features that contribute to the binding selectivity. The emergence of experimental techniques suitable for the determination of the DNA-binding selectivity, such as footprinting, coupled with high-resolution definition of structural details of binding to such sequences via X-ray crystallography, NMR and molecular modeling, have advanced the understanding of drug-DNA interactions and the nature of the chemical reactions provided by such agents on DNA. This knowledge permitted the synthesis of selected targets with good in vitro and in vivo properties. This contribution will be focused on the synthesis and activity of some heterocyclic analogs of three classes of minor-groove binders, such as anthramycins, CC-1065 and distamycins. In particular, the bioisosteric replacement of the native nucleus with different heterocycles will be examined.

2. Anthramycin derivatives

In 1963, anthramycin (**1**), the first term of a new class of natural compounds called pyrrolobenzodiazepines (PBDs), was discovered [1–3].



Anthramycin **1**

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* Corresponding author. Tel.: +39-0532-291293; fax: +39-0532-291296.

E-mail address: pgb@ifeuniv.unife.it (P.G. Baraldi)

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This natural compound is an antitumor antibiotic, isolated from the fermentation broth of the termophilic actinomycete *Streptomyces refaineus* [4]. It has been isolated, characterized and synthesized by several groups establishing that: (a) PBDs bind to double stranded DNA, but not to RNA; (b) a covalent bond is formed between drug and DNA; (c) the PBD family do not belong to the intercalators group, because of an approximately 36° molecular twist due to asymmetric atoms C11 and C11a; and (d) PBDs form a bond with N2-guanine residues [5–8].

The molecule consists of a tricyclic system, namely an aromatic A-ring, a pyrrolidine C-ring and a 1,4-diazepin-5-one bearing a N10–C11 iminecarbinolamine moiety.

All natural occurring compounds possess the (*S*) configuration at C11a, which provides the molecules with a right-handed twist when viewed from the C-ring towards the A-ring. This provides the isoelicity with the DNA minor-groove needed for the docking (in fact, racemization at C11a significantly reduces the biological activity) [9–11].

The reaction site N10–C11, responsible for binding to DNA, can exist in at least three different interchangeable forms (Fig. 1), all of them possessing the ability to alkylate N2–guanine residues: imine (**2**), carbinolamine (**3**) and carbinolamine methyl ether (**4**).

Three mechanisms may be envisaged for reaction of these species with the 2-amino functionality of guanine (Fig. 2). One route (a) might proceed via a direct S_N2Ca-type attack of the protonated carbinolamine **6** (or its 11-methyl ether **7**) by the biological nucleophile, affording the adduct **9** [12]. A second mechanism (b) might involve Schiff's base formation between N2-guanine functionality and the acyclic amino aldehyde of DNA-drug adduct **8** [13], followed by intramolecular cyclization via attack of the aromatic amino function. The third mechanism (c) could be due to a direct attack of the biological nucleophile on the imine function of type **2** [14].

The ranking of binding preferences, as predicted by a theoretical model, is as follows:

$$A–G–A > A–G–G > G–G–A > G–G–G$$

Anthramycin (**1**) has been shown to have broad antitumor activity against a variety of transplanted tumors, e.g. Ehrlich solid carcinoma, sarcoma 180, human epidermoid carcinoma no. 3, leukemia L1210 cells [15–19].

Despite these activities, the clinical use of anthramycin has been limited by a high cardiotoxicity, and early workers also noted acute tissue necrosis at the site of injection [20–23]. These effects are closely related to those of anthracyclines (doxorubicine and adriamycine), due to radical formation during quinone–hydroquinone conversion.

Since PBDs lack quinone structures, the tautomerization or oxidation of anthramycin leading to *ortho*-quinone imine products has been postulated (Fig. 3). For this reason, several groups have focused their attention on the synthesis of heterocyclic PBD analogs. In fact the substitution of hydroxyl functions, present in natural compounds in the 9 position, with intraanular nitrogens, could confer to the molecule the same biocharacteristics

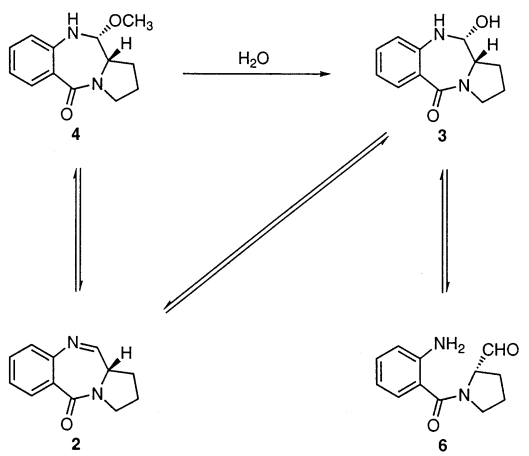


Fig. 1. Possible forms of PBDs that can alkylate DNA.

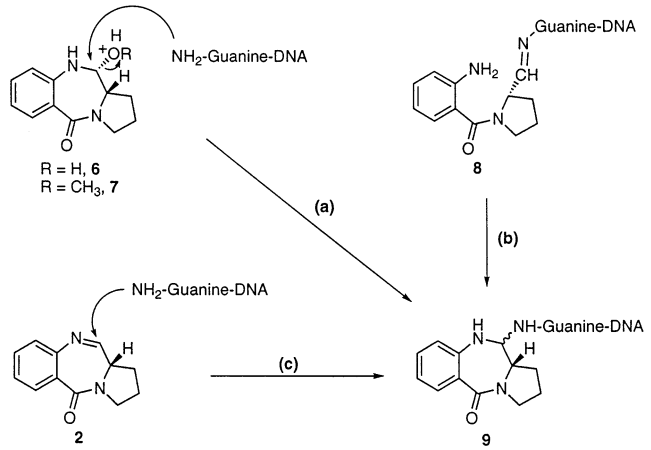


Fig. 2. Possible mechanism of DNA alkylation of PBDs.

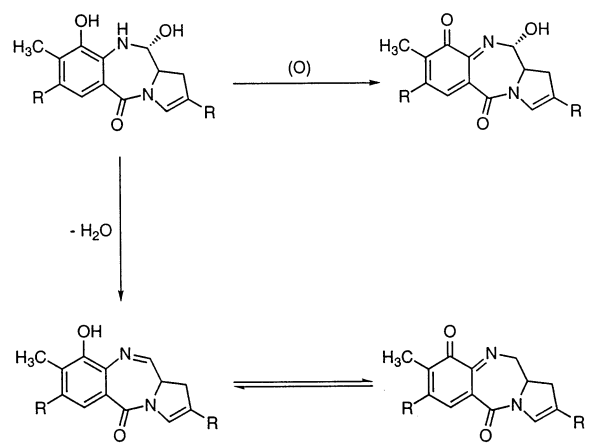


Fig. 3. Postulated mechanisms for the formation of quinoneimines.

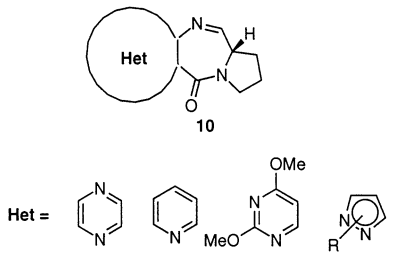


Fig. 4. Heterocycles utilized for bioisosteric modification.

of the parent compounds, avoiding the formation of quinone–imines responsible for cardiotoxicity [12]. A variety of A-ring-heterosubstituted PBD analogs of general formula **10** have been reported by our group in the last few years (Fig. 4) [24–28].

The rationale for the synthesis of these compounds is based on the following features: (a) preserve cytotoxicity against tumor cell lines; (b) obtain compounds with low cardiotoxicity; and (c) modulate the reactivity of carbinolamine (or its equivalent).

On this basis, we can summarize the results obtained with the hetero-PBDs described in Fig. 5.

In the pyrazolic series, some of the synthesized compounds showed a cytotoxicity against L1210 leukemia cell lines comparable to DC-81 **11** (μM range) [24–26]. In particular it has been observed that:

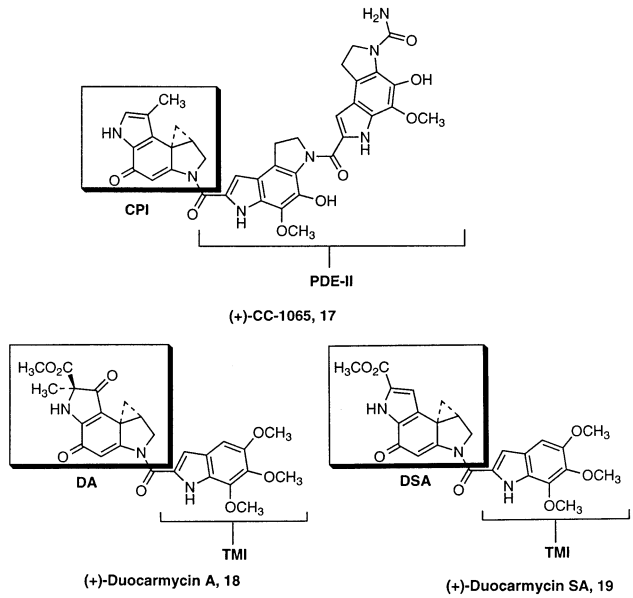
1. N7 substitution increases cytotoxicity with respect to N6 substitution
2. within substituents on the pyrazole ring, N7-benzyl or N7-substituted benzyl derivatives show superior activity towards leukemia cell lines
3. sterically hindered groups at the C8 position on the pyrazole ring cause a decrease of in vitro activity, in analogy to the C9 position on 'classic' PBDs
4. in in vivo studies, all these derivatives showed low activity, but cadiotoxicity was absent too, confirming the fact that the formation of radicals via quinoneimine is connected to heart damage.

As far as derivatives **14–16** are concerned, a significant decrease of activity (10–100-fold less active) was observed with respect to pyrazolic analogs and DC-8 1 [27,28]. These results suggest that the presence of electron withdrawing nitrogens in the A ring of PBDs produces an increase in the reactivity of carbinolamine, which can react with non-specific endogenous nucleophilic agents, with a consequent loss of DNA-specificity. On the basis of these experimental observations, we suggested that an increase of carbinolamine reactivity is detrimental in terms of cytotoxicity. Therefore, A-ring modifications do not boost PBD activity as much as C-ring modifications.

The combination of heterocyclic A-ring analogs with side chains at the C-ring could lead to obtaining new potent derivatives with no cardiotoxic side effects.

3. CC-1065 derivatives

CC-1065 (**17**) [29,30] and duocarmycins (**18, 19**) [31,32] are members of a class of exceptionally potent antitumor antibiotics that derive their biological effects through the reversible, stereoelectronically-controlled sequence of selective alkylation of DNA [33,34].



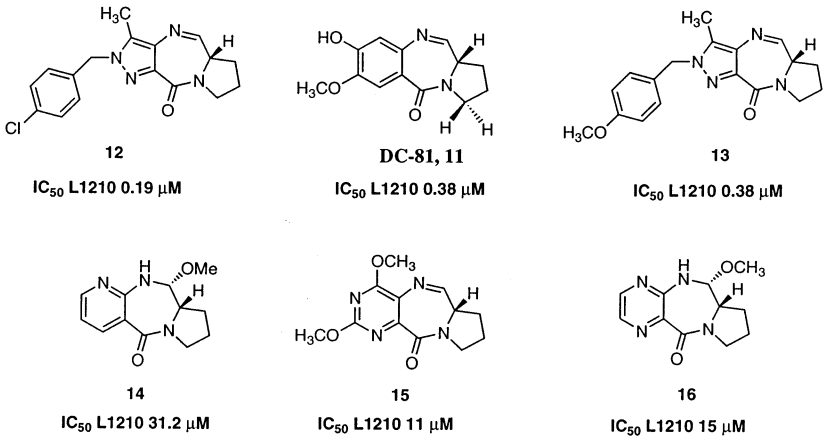
The phenomena of DNA alkylation for **17–19** involve the formation of a covalent bond between the unsubstituted carbon of the cyclopropane ring and the N3-adenine atom [33] (Fig. 6). This alkylation mechanism has been confirmed by full characterization of the released adduct DNA-agent after depurination.

All these compounds showed a high sequence selectivity for A–T regions. In particular, **17** reacts with two preferred reaction DNA sequences, constituted of five base pairs, being identified: 5′-PuNTTA* and 5′-AAAAA* where the compound in each sequence reacts at the 3′ (asterisked) adenine, Pu is purine (adenine or guanine) and N any base. Instead, duocarmycins (**18, 19**) preferred sequences constituted of three base pairs with the following order:

$$5'\text{-AA}\underline{\text{A}} > 5'\text{-TT}\underline{\text{A}} > 5'\text{-TA}\underline{\text{A}} > 5'\text{-AT}\underline{\text{A}}$$

As mentioned above, all these compounds provide predominantly adenine N3-adduct; a minor guanine N3-alkylation has been detected but only upon isolation of the thermally released adduct following treatment of DNA with an excess of agent [34–36].

All natural compounds showed cytotoxicity against leukemia L1210 cell lines in the pM range (10–220) but, while CC-1065 had a good antitumor activity in the in vivo model (optimal dose from 10 to 100 μg/kg), duocarmycins show weak antitumor activity [37]. De-



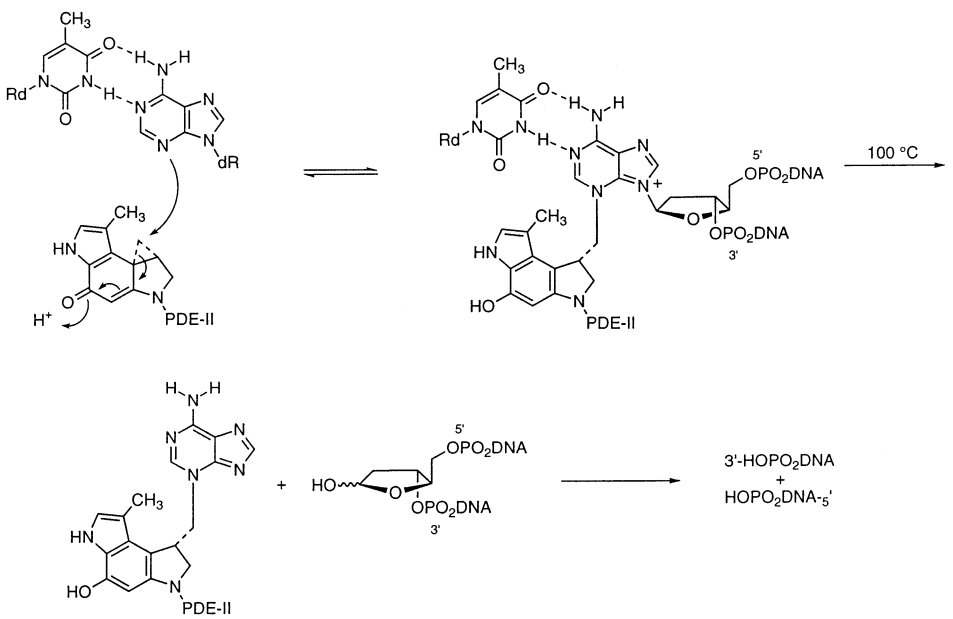


Fig. 6. Course of alkylation by **69**.

spite its potency, CC-1065 cannot be used in humans, because it induces delayed death [38]. This effect is accompanied by dramatic changes in the hepatic mitochondria morphology. For these reasons many scientists have focused their attention on this class of compounds, in order to obtain new derivatives with equal in vitro potency but a better profile in in vivo models.

In the last two decades, great progress has been made by scientists of different institutions, in understanding and exploiting the potential of CC1065 and its analogs. Information regarding the structural features required for activity are summarized in Fig. 7 [37].

On this basis, some interesting compounds have been synthesized and could be considered clinical candidates

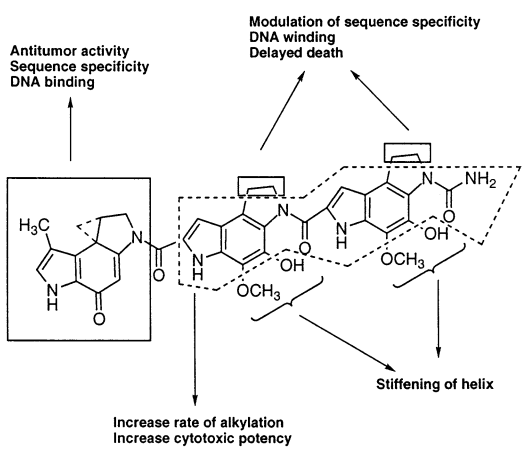
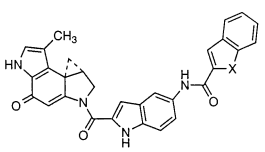


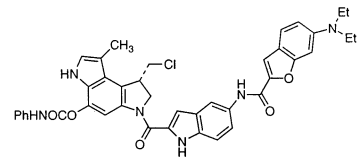
Fig. 7. CC-1065 structure–activity relationship.

for tumor treatment [39,40]. In particular four agents (**20–23**), analogs of **17**, containing the CPI alkylation subunit or a seco-precursor, have been chosen as clinical candidates.

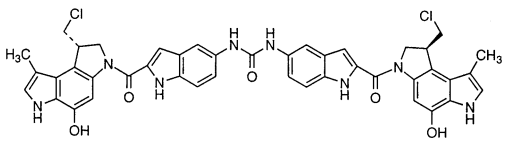
The first two, named U-71,184 (**20**) and adozelesin (**21**) [41], were derived from the first stage of studies on CC-1065 analogs, in which systematic simplifications of the DNA binding subunit were conducted. Carzelesin (**22**) is a prodrug that, after carbamate hydrolysis, cyclizes to the corresponding spiro derivative, containing cyclopropane [42]. Bizelesin (**23**), is a cross linking agent, incorporating two CPI units as seco-derivative and is 20–30 times more active than **17** (IC_{50} against L1210 = 1 pM) [43].



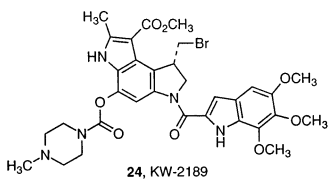
X = NH, **20**, U-71,184
X = O, **21**, Adozelesin



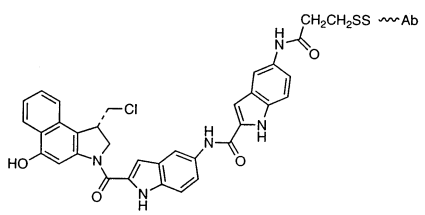
22, Carzelesin



23, Bizelesin



24, KW-2189



25, *anti*-B4-DC1

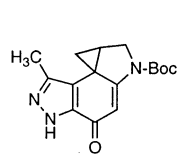
From the duocarmycin family, KW-2189 (**24**), which possesses improved antitumor activity and water stability, has been selected for clinical trials in Japan [44]. Boger and co-workers have recently reported a CBI-derivative (**25**) linked to humanized versions of the antibodies (mAbs) anti-B4 and N901 via a cleavable disulfide. This derivative showed in vivo antitumor efficacy in an aggressive, metastatic, human B-cell lymphoma model, and completely cured animals bearing large tumors [45].

In order to establish the steric and electronic parameters necessary for biological activity, some groups have focused their attention on the synthesis of heterocyclic analogs of CC-1065 and duocarmycins. The rationale behind the bioisosteric substitution of the pyrrole ring (present in natural compounds) with different heterocycles is based on the modulation of reactivity of the cyclopropane ring reactivity, which is responsible for DNA alkylation. Furthermore, this substitution could confer to the dimer or trimer agents different properties in in vivo studies. Our group has recently reported the synthesis and the biological evaluation of some pyrazole analogs (**26, 27**) of *N*-Boc-CPI [46–49].

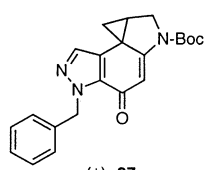
Table 1
Cytotoxicity and solvolytic stability of heterocyclic analogs of CPI and reference compounds

Compound	K (s^{-1}, pH 3)	$t_{1/2}$ (h, pH 3)	IC_{50} (L1210) (nM)
(+)-*N*-Boc-CPI	5.26×10^{-6}	37	330
(+)-*N*-Boc-DSA	1.08×10^{-6}	177	6
(±)-*N*-Boc-CPzI, **26**	1.75×10^{-6}	110	370 (185)[a]
(±)-**27**	9.082×10^{-7}	212	3064 (1532)[a]

[a] Predicted values in parentheses.



(±)-N-Boc-CPzI, **26**

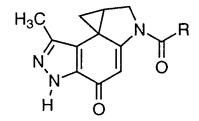


(±)- **27**

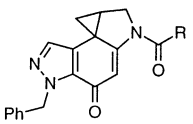
Both derivatives (**26, 27**) have been tested against L1210 leukemia cell lines, and their solvolytic stability to pH 3 has been examined (Table 1). It is remarkable that both compounds showed a solvolytic stability higher than that of CPI but comparable cytotoxicity with respect to the reference compound. Even if it is not accurate to compare a racemic mixture and a pure enantiomer, on the bases of enantiomeric distinction of CPI analogs it is possible to assume that the unnatural enantiomer is $\geq$10-fold less potent than the natural enantiomer and, consequently, it is reasonable to attribute an IC_{50} of about 185 nM for **26** [47–49]. This theoretical value would be ca. 2-fold more potent than (+)-*N*-Boc-CPI and very close to that expected on the basis of a direct relationship between chemical solvolytic stability and cytotoxicity, as proposed by Boger et al. [45]. A different explanation should be given for derivative **27**. In fact, if even we made the assumption mentioned above, the derivative would have an IC_{50} of about 1532 nM. This loss of activity could be attributed to the presence of the benzyl group on the pyrazole nitrogen, because the free N–H seems to play a fundamental role for the anticellular activity [50].

Dimer and trimer agents have been prepared for both alkylating subunits (**28–32**), utilizing 'carriers' present

in the compounds actually under clinical investigation, such as U-71,184 (**20**) and adozelesin (**21**) [48,51].



R = Indole$_2$, **28**
R = Indole-Benzofurane, **29**



R = Indole$_1$, **30**
R = Indole$_2$, **31**
R = Indole-Benzofurane, **32**

Cytotoxicity against L1210 leukemia cell lines has been evaluated for all compounds (Table 2). Compounds **28–32** showed cytotoxicity values in the pM range. These results confirm the relevance of non-covalent interactions in DNA binding. This observation is also evident when comparing cytotoxicity of trimer agents **28**, **29**, **31**, and **32** with respect to compound **30**, which bears a simplified 'carrier'; the latter proved to be less active by about 30-fold.

All of the derivatives were about 10–100-fold less cytotoxic than the parent drugs adozelesin (**21**) and U-71,184 (**20**). This difference could be explained with the already mentioned enantiomer distinction existing between the (+) and (−) forms of CPI derivatives. In fact, these values are comparable with the data reported for the racemic forms of **20** and **21**.

Synthesized compounds also showed a good cytotoxicity against multidrug resistant L1210/Dx subline. Furthermore, the effectiveness against L1210 leukemia of compounds **29–32** following intravenous (i.v.) administration has been reported and summarized in Table 3 [48,51]. While compound **29**, when given as a single i.v. injection 1 day after tumor inoculation (i.v.), proved to be active against L1210 leukemia at 50–100 μg/kg (Tables 4 and 5), with a low toxicity, derivatives **30–32** possess good potency (300 μg/kg) but no activity, with a ratio of survivors/total of 0/10.

These results confirm the hypothesis that the presence of a hydrophilic function (pyrazole nitrogen) confers to the drug a higher stability in water solution, and consequently a better distribution to the tumor in treated animals as observed from our group and Upjohn. An increase of the hydrophobic portion gives the opposite effect [37].

Table 2
In vitro cytotoxicity of adozelesin analogs against L1210 leukemia cells

Compound	IC_{50} L1210 (pM)
20, U-71,184	4
21, Adozelesin	3.9
(±)-**20**	10
(±)-**21**	20
(±)-**28**	36
(±)-**29**	28
(±)-**30**	1736
(±)-**31**	52
(±)-**32**	70

Table 3
Comparative antileukemic activity against disseminated L1210 leukemia of **29–32**

Compound	O.D. (μg/kg)	ILS (%)	Survivors/total
(±)-**29**	100	363	4/10
(±)-**30**	300	17	0/10
(±)-**31**	300	15	0/10
(±)-**32**	300	11	0/10

Table 4
Antileukemic activity against disseminated L1210 leukemia of (±)-**29**

Dose (μg/kg)	ILS (%)	Survivors/total	Toxicity
50	169	2/10	0/10
100	363	4/10	0/10
200	0	1/10	8/10

Table 5
Comparative antileukemic activity against disseminated L1210 leukemia of adozelesin (**21**) and (±)-**29**[a]

Compound	O.D. (μg/kg)[b]	ILS (%)[c]	Survivors/total
Adozelesin, **21**	100	221	2/6
(±)-**29**	100	363	4/10

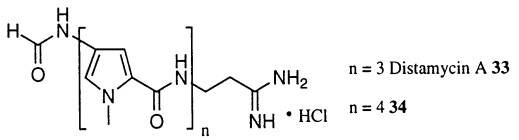
[a] L1210 murine leukemia: 10^5 cells/mouse were injected i.v. on day 0; treatment was given i.v. on day 1 after tumor implantation.
[b] O.D., optimal dose i.e. the dose producing the greatest efficiency and having an acceptable level (≤10%) of toxicity.
[c] ILS, percent increase in life span: [(MST of drug-treated group/MST of vehicle-treated control mice) − 1] × 100.

4. Distamycin derivatives

The tripyrrole peptide distamycin A **33** [52] is a naturally occurring antibiotic agent isolated from the cultures of *Streptomyces distallicus*. It showed interesting antibacterial and antiviral activities (yet was inactive as an antitumor agent) [53], and proved to bind exclusively to the DNA minor-groove by means of hydrogen bonds, van der Waals contacts and electrostatic interactions. This oligopeptide shows a high selectivity for AT rich sequences, covers five base pairs and binds preferentially to 5′-AAATT-3′ sequences [54].

The presence of the C2 amino group of a guanine in GC base pairs introduces a steric hindrance to ligand binding in GC-containing sequences, but the replacing of a pyrrole ring with an imidazole in distamycin A transforms the steric interference into an energetically-favorable hydrogen bond. For this reason, a novel class of minor-groove binding molecules called 'lexitropsins', or information-reading oligopeptides, in which one or

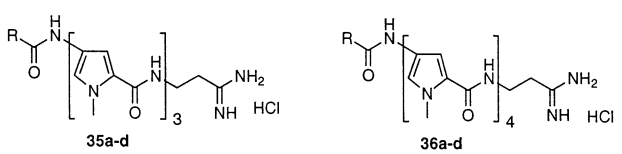
more pyrrole rings have been replaced with other heterocyclic moieties (e.g. imidazole or thiazole), have yielded ligands with an increased tolerance for GC base pairs at their binding sites [55]. The tetrapyrrole distamycin A homolog **34**, although exerting very low cytotoxic activity, is almost 20-fold more active than the parent compound **33** [56]; it has been highlighted that by increasing the number of *N*-methylpyrrolecarboxamides, the sequence specificity for longer tracts of AT-rich DNA has been achieved [57].

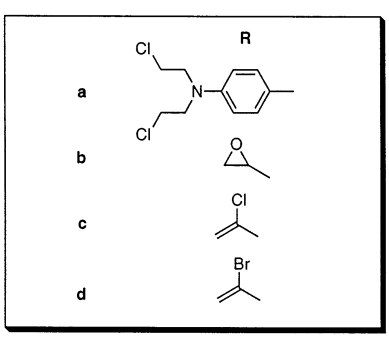


It is widely known that distamycin A has been used as vector for alkylating functionalities such as mustard analogs, which have been reported to form guanine N7 adducts within the DNA-major groove [58].

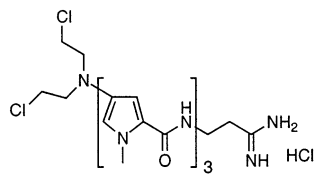
The distamycin A derivative tallimustine **35a** (FCE 24517), in which the formyl group has been substituted by a benzoyl nitrogen mustard moiety, is a potent antitumor agent, active against several murine tumors, and is now in clinic Phase II [59]. This compound retains the AT preference of distamycin A and seems to possess a high preference for alkylation of the 3′-adenine-N3 atom located in the sequence 5′-TTTTGA-3′ [60]. The potent antitumor activity of tallimustine may be due to its ability to inhibit the binding of not yet characterized protein factor(s) which recognize this sequence of the DNA. It is worth noting that the cytotoxicity can be improved by increasing the number of pyrrole residues from three (as in tallimustine) to four in **36a**, and that a relationship has been demonstrated between the number of pyrroles and cytotoxicity [56].

A large series of distamycin A derivatives bearing other different alkylating moieties (such as epoxycarbonyl, halogenoacriloyl or nitrogen mustard) on the N-terminal position with three or four pyrrole rings has been synthesized [61].





All the compounds in this series show much higher activity than that of distamycin. As previously reported for tallimustine and its homolog **36a**, in each class of derivatives it has been observed that by increasing the number of pyrrole rings an increase in in vitro cytotoxicity and in vivo potency on L1210 murine leukemia cells can be envisaged. The mustard derivative of distamycin **37** showed 3-fold more activity than the parent compound distamycin A against the KB cancer cell line.



37

The most active compound **36d**, a tetrapyrrole possessing the α-bromoacryloyl alkylating moiety, is approximately 1000 times more potent than distamycin A and 5-fold more cytotoxic than tallimustine (Table 6) [56,62]. By modifying the amidine side chain of tallimustine analogs with various aminoalkylamidines no significant improvement in their biological activities has been detected [63].

These interesting findings prompted us to synthesize new distamycin derivatives in which the pyrrole ring bearing the alkylating moiety (benzoyl nitrogen mustard or α-bromoacryloyl moieties) has been replaced by isosteric imidazole or pyrazole rings [64].

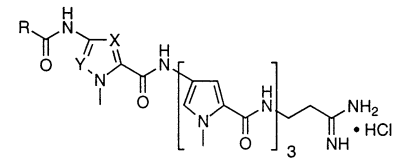
The cytotoxicity of the alkylating tetrapeptides (tested both in vitro and in vivo on L1210 murine leukemia), with the notable exception of imidazolic benzoyl nitrogen mustard **38**, was much greater than that of the parent distamycin A. Moreover, we were interested in comparing the cytotoxic activity of com-

Table 6
Cytotoxicity and antitumor activity on L1210 murine leukemia

Compound	In vitro	In vivo	
	IC_{50} (ng/ml)[a]	O.D. (mg/kg)[b]	% T/C[c]
30	5216.0	200	113
31	285	n.d	n.d
35a	24.4	3.125	175
35b	229	50	188
35c	56	10	184
35d	49.6	12.5	175
36a	16.3	0.39	138
36b	38.9	3.125	188
36c	2.7	3.125	171
36d	4.7	3.125	206
37	2.17	1.56	138

[a] IC_{50}, 50% inhibitory concentration represents the mean from dose–response curves of at least three experiments.
[b] O.D., optimal dose; optimal non toxic dose < LD10.
[c] % T/C, median survival time of treated vs. untreated mice × 100.

pounds **38–41** with tallimustine **35a**, and in contrast with the poor activity shown by **38**, its pyrazole analog **39** showed significant cytotoxicity comparable to that of tallimustine.



R = $(ClCH_2CH_2)_2N$— **38**

Y=CH , X=N

R = $(ClCH_2CH_2)_2N$— **39**

Y=N , X=CH

R =  **40**

Y=CH , X=N

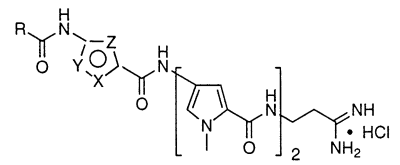
R =  **41**

Y=N , X=CH

The poor activity of **38** might be related to a different binding pattern to DNA recognition sequence, due to a possible role of the N(3) lone pair of the imidazole ring (Table 7). The kind of alkylating group had a great effect on the cytotoxicity of compounds sharing the same oligopeptide frame. Compound **40**, bearing an α-bromoacryloyl moiety, was at least 60-fold more potent that the benzoyl mustard counterpart. The derivatives bearing an α-bromoacryloyl moiety, both with pyrazole and imidazole, appeared of relevant interest as they proved to be more potent than tallimustine. In general, by comparing the tetraoligopeptides bearing the same alkylating moiety and a different N-terminal heterocycle, it can be observed that the pyrrole nucleus conferred an antiproliferative activity which was much higher than that of pyrazole and imidazole.

Tallimustine analogs in which one or more pyrrole moieties were replaced with a different heterocyclic system are interesting both for the discovery of more potent anticancer agents and for the peculiar DNA sequence specificity. At the beginning, we studied the effect of the replacement of the N-terminal pyrrole ring with other heterocyclic moieties as pyrazole, imidazole or thiazole, the latter two showing increased tolerance for GC base pairs.

All the derivatives **42–46** were evaluated both in vitro and in vivo against L1210 murine leukemia, and the compounds **42–45** turned out to be 700-, 30-, 40- and 500-fold less active in vitro than tallimustine, respectively.



X=CH Y=S Z= N **42**

R = $(ClCH_2CH_2)_2N$—

X= N-CH_3 Y= N Z= CH **43**

R= $(ClCH_2CH_2)_2N$—

X=N-CH_3 Y=CH Z=N **44**

R = $(ClCH_2CH_2)_2N$—

X=CH Y=S Z= N **45**

R= 

X= N-CH_3 Y= N Z= CH **46**

R= 

Introduction of different alkylating functions did not improve the antitumor activity of the thiazole derivative **45**, whose activity remains comparable to that of benzoyl nitrogen derivative **42**. On the other hand, the substitution of the benzoyl nitrogen moiety with an α-bromoacryloyl unit in the monopyrazole analog **46**

Table 7
Cytotoxicity and antitumor activity of compounds **38–41** on L1210 murine leukemia

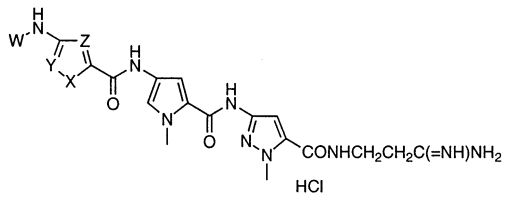
Compound	In vitro	In vivo	
	IC_{50} (ng/ml)	O.D. (mg/kg)	% T/C
33	5216.0	200	113
35a	24.4	3.125	175
38	2000	6.25	125
39	29.1	3.13	144
40	35	6.25	163
41	9.9	6.25	200

Table 8
Cytotoxicity and antitumor activity on L1210 murine leukemia of compounds **42–49**

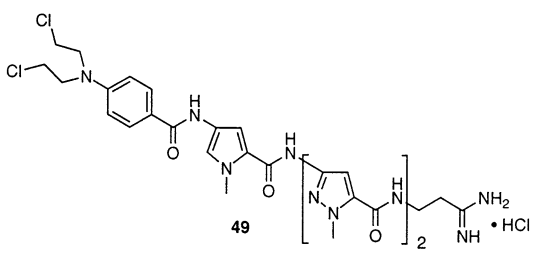
Compound	In vitro	In vivo	
	IC_{50} (ng/ml)	O.D. (mg/kg)	% T/C
33	5216.0	200	113
35a	24.4	3.125	175
42	32463	n.d	n.d
43	1390	12.5	118
44	1920	6.25	100
45	23820	n.d	n.d
46	151	30	181
47	7216	n.d	n.d
48	35	6.25	213
49	112	25	259

proved to be effective in increasing the cytostatic activity, being only three-times less active than tallimustine (Table 8). This further confirmed the hypothesis that, for the same oligopeptide chain, the α-bromoacryloyl moiety gives better results in terms of antileukemic activity in comparison to the benzoyl mustard [65].

There are other examples of tallimustine analogs tethering a benzoyl nitrogen mustard, where the N-terminal pyrrole bearing the alkylating moiety is replaced with thiazole residue and one or two pyrrole nuclei close to the amidine function are replaced by pyrazole rings.



47 X=S Y=C Z=N
48 X=N-CH_3 Y=Z=CH

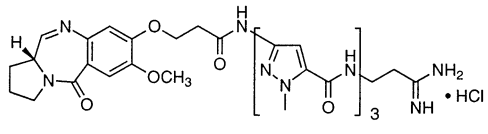


49

Compound **48**, in which the last *N*-methyl pyrrole ring of tallimustine was replaced by *N*-methyl pyrazole, showed the same cytotoxicity of tallimustine and was less toxic in vivo (O.D. = 6.25 versus 3.13 mg/kg) with an increased survival time (% T/C). On the other hand, the replacement of two pyrrole units with two pyrazoles, as in compound **49**, led to a 5-fold decrease of activity. Derivative **47**, bearing both thiazole and pyrazole rings, showed a relevant decrease of the activity in comparison with pyrrole or pyrazole analogs.

5. Perspectives

In recent times [66], many efforts have been aimed at combining different classes of antitumor agents into so called hybrid molecules, on the hypothesis that, by mixing the DNA recognition site preferences of each class, these new drugs might interact with 'sensitive' regions of nucleic acids, such as the oncogenes. Our 'know-how' on distamycins and anthramycines prompted us to explore a combination of distamycin A with a PBD derivative, tethered together by means of a five-atom chain (**50**).



50

Cell growth inhibition (K562) IC_{50} 0.2 μM

The new hybrid, as expected, preserved both A–T selectivity, peculiar to distamycins, and the G–C binding site of anthramycins, and most interestingly, it propelled the binding time from the hours (as in the anthramycin class) to the minutes/seconds range, probably due to the polypyrrole carrier acting as a DNA recognition moiety [67].

This finding could represent a new approach in the discovery of antitumor agents because these hybrid molecules could possess not only high potency but also different alkylation sites, both conditions being indispensable for tumor treatment. Furthermore, as in the case of carriers possessing hydrophilic moieties, such as distamycin, the water solubility of the compound could be increased facilitating the parenteral administration.

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