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Review

Hybrid molecules between distamycin A and active moieties of antitumor agents

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Abstract—The DNA minor groove is an attractive target for the design and development of molecules able to specifically recognize predetermined DNA sequences. The pyrrole-amide skeleton of distamycin A has been also used as DNA sequence selective vehicle for the delivery of alkylating functions to DNA targets. Selectivity for specific sequences may be of particular importance in affecting the activity of regulatory genes (oncogenes and tumor suppressor genes). Recent work on a number of hybrid compounds, in which known antitumor compounds or simple active moieties of known antitumor agents have been tethered to distamycin frame or hairpin polyamides derived from distamycin, is reviewed. The DNA alkylating and growth inhibition activities against several tumor cell lines are reported and discussed in terms of their structural differences in relation to both the number of *N*-methyl pyrrolic rings and the type of the alkylating unit tethered to the oligopyrrolic frame.

Contents

1. Introduction

Many natural and synthetic anticancer agents with the ability to interact with DNA have been discovered, but most have little sequence specificity and often exhibit severe toxicity to normal tissues. In the discovery of novel gene-based therapeutic agents, DNA minor groove binders have constituted an attractive source of novel antitumor molecules. The increasing interest in this group of compounds stems from their ability to interact in a sequence-selective fashion at quite long DNA-binding sites, suggesting the possibility of targeting specific DNA sequences within the genome. These molecules are frequently based on natural products and have been investigated for their ability to interact selectively with the minor groove of DNA.[1] One of the most studied minor groove binders is distamycin A.

Distamycin A (**1**) is a naturally occurring antibiotic agent isolated from *Streptomyces distallicus* active against some viruses, Gram-positive bacteria and protozoa (but inactive as antitumor agent).[2] Distamycin A is characterized by the presence of an oligopeptidic pyrrolecarbamoyl frame ending with an amidino moiety, with sequence specificity toward AT-rich sites within duplex DNA. Saturating the distamycin pyrrole rings, to obtain

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an oligopeptidic pyrrolidinecarbamoyl frame with three additional basic nitrogens, greatly reduces the DNA-binding affinity observed with distamycin A.[3]

Its sequence specificity and high affinity is derived from a combination of interactions including hydrogen bonding, van der Waals contacts, and electrostatic interactions of the cationic amidine side chain with the phosphate backbone of DNA.[4] Distamycin can also inhibit protein interactions with G-quadruplex (G4) DNA, a stable four-stranded structure in which the repeating unit is a G-quartet.[5] The specific stabilization of AT-rich DNA–DNA duplexes by distamycin was preserved upon conjugation with oligodeoxyribonucleoside (ODN) stretches.[6]

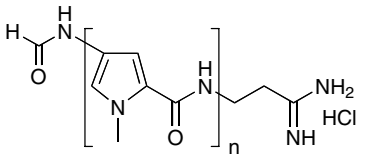
Distamycin A can bind to DNA duplexes containing the (6-4) photoproduct (one of the major ultraviolet lesions in DNA) despite the changes, caused by photoproduct formation, in both the chemical structure of the base moiety and the local tertiary structure of the helix. A 20-mer duplex containing the target site, AATT–AATT, was designed, and then one of the TT sequences was changed to the (6-4) photoproduct. Curve fitting of the CD titration data revealed that the binding stoichiometry changed from 1:1 to 2:1 with photoproduct formation.[7]

It is possible to achieve high affinity and selectivity of DNA binding by the distamycin A analogue **2**, which contains 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. Two molecules bind side-by-side in the minor groove, but their binding is staggered so that the molecule reads six base pairs, unlike the related natural products, which tend to bind to four-base-pair sequences.[8]

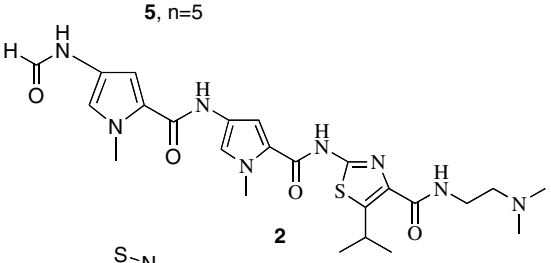
Starting from compound **2**, with the aim of preparing lipophilic analogues of distamycin with enhanced membrane permeability for the microbes, significant antimicrobial activity is clustered around a small number of structural features, namely branched *N*-alkylpyrroles, hydrophobic *N*-terminal amides, and especially *C*-isopropylthiazoles. Significant antimicrobial activity against key organisms such as MRSA and *Candida albicans* is shown by several compounds, especially those containing a thiazole. Moreover, these compounds have low toxicity with respect to several mammalian cell lines.[9]

The three *N*-methyl pyrrole carboxamide units of distamycin A linked at the N-terminal position to an isothiazole ring furnished a small library of compounds (with general formula **3**) consisting of a four-ring core element and characterized by the presence of various substituents at both termini. These compounds showed excellent activity against a broad spectrum of Gram-positive bacteria. The antifungal activity appeared to strongly depend on the basicity of the compounds; where the less basic molecules showed significantly reduced antifungal activity.[10]

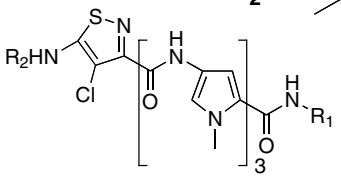
The four pyrrole distamycin A homologue **4** is almost 20-fold more potent than distamycin A and increasing the number of pyrrolic units of the oligopeptidic frame



1 (Distamycin A), n=3
4, n=4
5, n=5



2



3

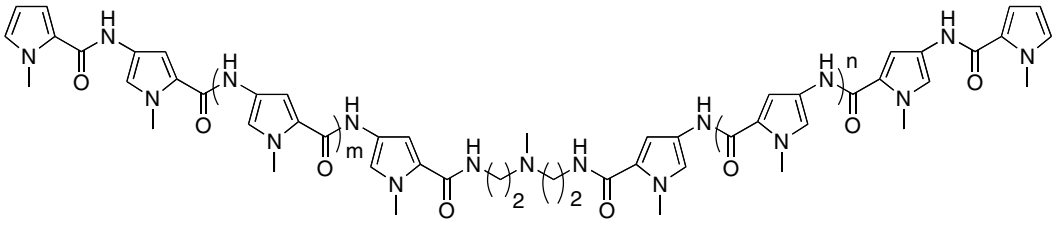
R_1= $(CH_2)_nN(CH_3)_2$ with n=3, 4 and 6; ethylmorpholine, ethylpyridine
R_2= $(CH_2)_nNH_2$ with n=3-5; ethylmorpholine, ethylpyridine

increases the sequence specificity for longer tracts of DNA AT-rich sequences, as a result of the greater availability of hydrogen bonding and van der Waals surface.

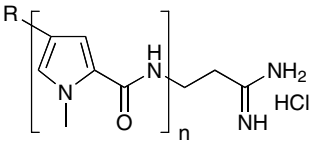
The five-pyrrole distamycin A derivative **5** acts as a telomerase inhibitor. Exposure of human melanoma cell extracts to compound **5** induced a dose-dependent inhibition of telomerase activity, with an IC_{50} of 24 μM.[11]

The synthesis of a tris-pyrrole analogue of distamycin A **1** lacking the N-terminus formamide unit, 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.[12] Increasing the number of pyrrolic rings over six units produced molecules which are out of phase with DNA. To reset an optimal fit of long polyamides with the DNA-double helix, two distamycin-like cationic peptides have been connected by linking the C-terminus of the individual monomers (*tail-to-tail* linkage). The positive charge necessary for optimizing the DNA affinity and water solubility is provided by the presence of a tertiary amine based linker which remains protonated at physiological pH.[13] These dimeric lexitropsin derivatives **6a**–**c** also lack the leading formamide unit at the N-termini. The present systems form a novel class of minor groove binders that bind to DNA in a bidentate fashion and exhibit significantly greater affinity for DNA compared to the respective monomers. These bind poly(d(A·T)) in a nearly 2:1 overlapped fashion and individual molecules seem to cover 8–10 bp.

With the objective to identify novel promising candidates, distamycin A and its homologue **4** have been used as



6a, m=o, n=o
6b, m=0, n=1
6c, m=1, n=1



7, n=3 or 4

R= 4-[$(ClCH_2CH_2)_2N$]C_6H_4CONH, 4-[$(ClCH_2CH_2)_2N$]$C_6H_4CH{=}CHCONH$, $CH_2(O)CHCONH$, 4-[$(ClCH_2CH_2)_2N$]$C_6H_4(CH_2)_3CONH$, $(ClCH_2CH_2)_2N$, $CH_2{=}CXCONH$ with X=F, Cl or Br .

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 (with general formula **7**) endowed with relevant cytotoxic and antitumor activity than distamycin and **4** themselves, respectively.[14]

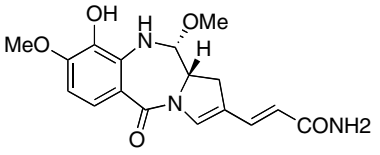
Hybrid compounds, in which known antitumor compounds or simple active moieties of known antitumor agents have been tethered to distamycin frame, have been extensively reviewed in the recent past.[15] The nature of antitumor agents and therefore also the rationale that led to these compounds were different. 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.

2. Hybrids between oligopyrroles structurally related to distamycin A and cytotoxics

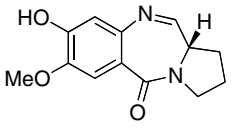
Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBD) group,[16] which includes the naturally occurring antitumor antibiotics anthramycin (**8**) and DC-81 (**9**), owes its DNA-interactive ability and resultant biological effects at the N10–C11 carbinolamine/imine moiety in the central B-ring which is capable of covalently binding to the C2–NH_2 of guanine residues in the minor groove of DNA. X-ray and footprinting studies on covalent DNA–PBD adduct have demonstrated a high sequence-specificity for GC-rich DNA regions, in particular for X-G-X triplets (X = purine).[17]

Both our[18,19] and Lown's group[20] have reported the synthesis, biological activity, and DNA-binding properties of novel hybrids (**10a**–**d**), consisting, respectively, of one, two, three or four pyrrole amide units linked to a pyrrolo[2,1-*c*][1,4]benzodiazepine **11**, through a spacer arm, in order to study the structure–activity relationship between length of the oligopyrrolic frame, antiproliferative activity, and sequence specificity.

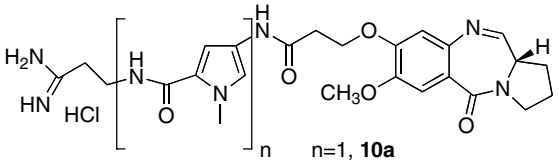
The rationale that led to the synthesis of this series of pyrrolo[2,1-*c*] [1,4]benzodiazepine-lexitropsin conjugates was to tether the distamycin A frame, which plays the role of pure minor groove binder, to the minor groove alkylating moiety represented by the pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) **11**, with the aim to obtain new derivatives which could become more cytotoxic than the parent compounds.



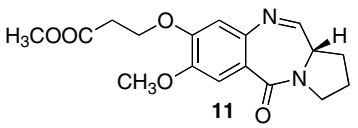
8, Anthramycin



9, DC-81



n=1, **10a**
n=2, **10b**
n=3, **10c**
n=4, **10d**



11

Table 1. In vitro biological effects of distamycin A (**1**), DC-81 (**9**), PBD methyl ester **11**, and PBD-polypyrrolic hybrids **10a–d** on K562 and Jurkat cell lines

Compound	IC_{50} (μM)	
	Jurkat	K562
Distamycin A (**1**)	20	12
DC-81 (**9**)	2.2	1
11	3	1.5
10a	80	>100
10b	50	6
10c	0.8	0.7
10d	0.07	0.04

IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.

The antiproliferative activity of the hybrids has been evaluated in vitro by using both the human chronic myeloid leukaemia K562 and the T-lymphoblastoid Jurkat cell lines. The results obtained are summarized in Table 1.

The results obtained demonstrate that the hybrids (**10a–d**) exhibit different DNA-binding activity with respect to both distamycin A **1** and PBD alkylating moiety **11**. With respect to antiproliferative effects, it was found that the increase in the length of the polypyrrole backbone led to an increase of in vitro antiproliferative effects, that is, the hybrid **10d** containing the four pyrrole distamycin analogue was more active than **10c** both against Jurkat (IC_{50} μM, 0.07 vs 0.8, respectively) and K562 (IC_{50} μM, 0.04 vs 0.7, respectively) cell lines. Only derivatives **10c** and **10d** retain a higher antiproliferative activity when compared to PBD **11** alone. In fact, compound **10a**, containing only one *N*-methylpyrrolic unit, showed negligible inhibitory activity or no activity at all. On the other hand, **10b**, containing two pyrrole moieties, exhibited to some extent antiproliferative activity on the K562 cell line (IC_{50} 6 μM), being scarcely active on Jurkat cells (IC_{50} 50 μM).

The tri- and tetrapyrrolic hybrids **10c** and **10d** are the most potent antiproliferative compounds of this series, exhibiting higher binding affinities with respect to the mono- and dipyrrolic conjugates **10a** and **10b**, probably due to additional amido hydrogen bonds and van der Waals interactions.

These data suggest that the higher antiproliferative activity of hybrid molecules containing pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) and minor groove oligopyrrole carriers containing three and four pyrrolic moieties is due to the recognition of additional binding sites than distamycin, as well as to an increase in the stability of drugs/DNA complexes. This is a reasonable hypothesis, since a tighter DNA binding could depend on the increased multiplicity of interactions between the increased number of pyrrolecarboxyl units and target DNA sequences.

The PBD–distamycin hybrid **10c** is also able to inhibit the DNA binding of the transcription factor Sp1.[21] The results obtained demonstrate that treatment of Sp1 target DNA with PBD–distamycin hybrid **10c** renders the site unrecognizable by nuclear proteins. These data are in our opinion of interest, since transcription factors belonging to the Sp1 superfamily are very important for the control of transcription of cellular and viral genes, including the oncogenes Ha-ras and c-myc, and the human immunodeficiency type 1 virus (HIV-1).[22]

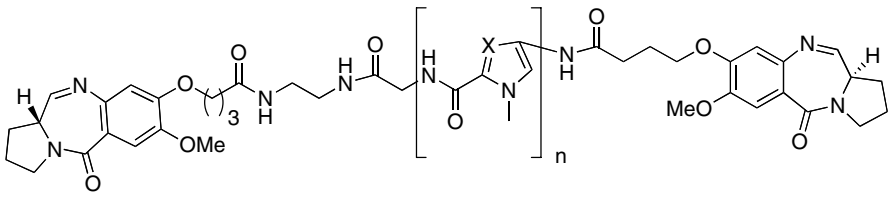
The hybrid compounds **10a–d** are also strong inhibitors of in vitro and ex in vivo transcription directed by the long terminal repeat (LTR) of HIV-1.[23] The higher inhibition of HIV-1 LTR-directed transcription by these hybrid molecules was observed for the derivatives with three and four pyrrolic moieties (compounds **10c** and **10d**, respectively) and it was mainly due to an increase in the stability of drugs/DNA complexes. With respect to the possible use of these compounds for therapeutic anti-HIV approaches, in vivo toxicity should be carefully analyzed. As we have reported before, unfortunately compounds **10c** and **10d** exhibit high antiproliferative activity, and therefore are expected to be toxic when administered to cells. However, compounds containing lower numbers of pyrrolic moieties could still exhibit biological activity, displaying at the same time low antiproliferative effects. In this context, the two-pyrrole hybrid **10b** appears of great interest, since it exhibits inhibitory effects on HIV-1-driven transcription, but low inhibitory effects on cell growth of Jurkat cells, and, therefore, could be proposed in further experiments (including bioavailability, gene expression profiling, and in vivo toxicity) aimed at developing novel anti-HIV agents.

The potential utility of the hybrids **10b–d** as future chemotherapeutics for the AIDS therapy was confirmed by their ability as RNA-binding drugs, which can interrupt protein/TAR-RNA interactions and Tat-induced LTR-driven HIV-transcription.[24] Tat is a regulatory protein that is required to induce high-level transcription of the HIV-1 genome after binding to a structured TAR-RNA. For this reason, inhibition of this complex assembly may represent a new target for anti-HIV compounds. Among the active molecules, compound **10b** appears to be of interest, since it exhibits the lowest cytotoxic profile.

Lown has recently reported the design, synthesis, and biological evaluation of novel pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) dimers (**12a–f**) linked with pyrrole and imidazole polyamides from either side by a flexible methylene chain of variable length (from 1 to 3). Both bis-PBD pyrrole (**12a–c**) and imidazole (**12e–f**) polyamide conjugates became potent against many human cancer cell lines.[25]

Our group has also evaluated the synthesis of a series of hybrids which represent a molecular combination of polypyrrole minor-groove binders structurally related to distamycin A and two pyrazole analogues of the left hand segment called cyclopropylpyrroloindole (CPI) of the potent antitumor antibiotic (+)-CC-1065.[26]

(+)-CC-1065 (**13**) is a member of the class of cyclopropylindole antitumor antibiotics extracted from cultures

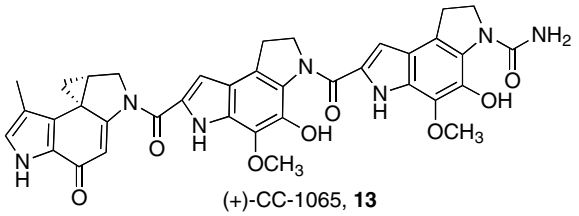


12a, n=1, X=CH
12b, n=2, X=CH
12c, n=3 X=CH
12d, n=1, X=N
12e, n=2, X=N
12f, n=3 X=N

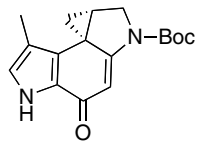
of *Streptomyces zelensis*[27] with activity both in vitro and in experimental animals.[28] Studies on the mechanism of cytotoxic action have shown that CC-1065 affords its biological activity through binding to DNA minor groove at AT-rich sequences, selectively alkylating at the N_3 position of the 3′-adenine by its cyclopropylpyrrolindole (CPI) subunit **14**.[29] Despite its high potency and broad spectrum of antitumor activity, CC-1065 cannot be used in humans because it causes delayed and irreversible toxicity in experimental models.[30]

Several years ago our group had synthesized two CPI pyrazole analogues named (±)-*N*-Boc-CPzI **15**[31,32] and (±)-*N*-Boc-*N*-BnCPzI **16**[33], which demonstrate a cytotoxicity against L1210 leukemia cells that was comparable to or 10-fold lower than, respectively, that of the reference compound *N*-Boc-CPI **14** [IC_{50} = 330 nM for (+)-*N*-Boc-CPI versus IC_{50} = 370 nM for (±)-**15** and IC_{50} = 3064 nM for (±)-**16**]. Because of their limited sequence specificity, low affinity for DNA, and poor water solubility, it was reasoned that it may be beneficial to tether these alkylating compounds to a DNA-binding vector, such as polypyrrole pseudopeptides, which can permeate cell membranes and has the potential to control specific gene expression. The vector could therefore deliver the reactive group more efficiently and in a sequence-specific manner to the DNA. Moreover, water solubility made these hybrid compounds attractive to overcome the administration problem of CC-1065 derivatives.

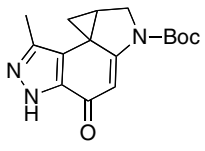
In synthesizing these novel water-soluble hybrids, we wanted to increase the potency of pyrazole CPI analogues **15** and **16** by increasing their affinity for DNA and to determine the structure–activity relationship between the length of the oligopyrrolic frame, antitumor activity, and sequence specificity.



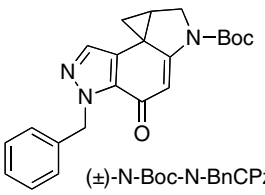
(+)-CC-1065, **13**



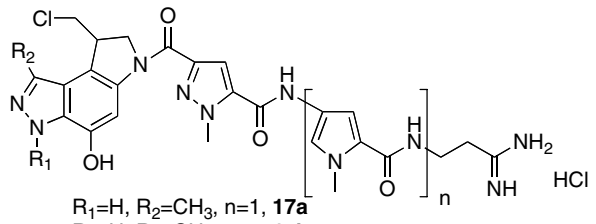
(+)-N-Boc-CPI, **14**



(±)-N-Boc-CPzI, **15**



(±)-N-Boc-N-BnCPzI, **16**



R_1=H, R_2=CH_3, n=1, **17a**
R_1=H, R_2=CH_3, n=2, **17b**
R_1=H, R_2=CH_3, n=3, **17c**
R_1=Bn, R_2=H, n=1, **17d**
R_1=Bn, R_2=H, n=2, **17e**
R_1=Bn, R_2=H, n=3, **17f**

Table 2. In vitro activity of alkylating units **15**–**16** and hybrids **17a**–**f** against the proliferation of five different cancer cell lines

Compound	IC_{50} (nM ± SE)				
	L1210	FM3A	Molt/4	CEM	Daudi
15	520 ± 6.6	1,400 ± 40	1,740 ± 50	1,260 ± 30	680 ± 150
16	2710 ± 490	18,300 ± 200	8,550 ± 280	6,720 ± 1,040	7,520 ± 30
17a	58+17	1600 ± 50	340 ± 20	230 ± 10	150 ± 40
17b	19 ± 2	190 ± 6	45 ± 1	39 ± 1	22 ± 10
17c	7.4 ± 0.4	31 ± 11	17 ± 4	71 ± 9	8.8 ± 0.1
17d	240 ± 30	4,000 ± 1000	130 ± 20	70 ± 21	11 ± 6.0
17e	600 ± 90	5600 ± 1400	160 ± 60	210 ± 110	38 ± 7.0
17f	400 ± 16	19,300 ± 3,400	310 ± 70	400 ± 50	100 ± 10

IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.

These hybrid compounds **17a**–**f** have been obtained by coupling the two *N*-Boc deprotected CPI pyrazole analogues **15** and **16** with three mixed pyrazole–pyrrole compounds called lexitropsins (or information-reading oligopeptides), consisting of a varying number of pyrrole amide units (from one to three) tethered on the N-terminus to a 3,5-pyrazole dicarboxylic acid moiety.

As evident from Table 2, it was found that tethering the pyrazole CPI analogues **15** and **16** to the DNA-binding lexitropsins afforded, with few exceptions, conjugate molecules that showed enhanced cytotoxic activity against five different cancer cell lines in vitro.

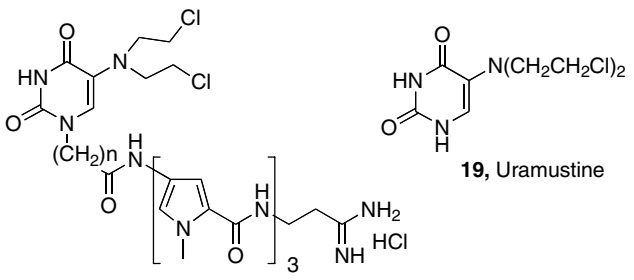
The results show that the hybrids **17a**–**f** were about 8- to 70-fold more potent than the alkylating unit **15**. Among these, the hybrid **17c** demonstrated the highest potency across the panel of tumor cell lines, especially against T- and B-lymphoblast cells, with IC_{50} values between 7.4 and 71 nM. Against L1210 cells, the tripyrrolic analogue **17c** was 2- to 8-fold more active than the mono- and bispyrrolic counterparts (compounds **17a** and **17b**, respectively). This is presumably due to the increased DNA binding of the 'longer' compounds. Compound **17c** was more active against L1210 cells than against the other tumor cell lines. For this series of hybrids it is possible to correlate structure with biological activity, increasing the number of pyrrolic rings from one to three results in increased cytotoxic activity.

The hybrids **17d**–**f** demonstrated potent cytotoxic activity against Daudi cells (IC_{50} values ranging from 11 to 100 nM). While being somewhat less toxic to the other tumor cells (IC_{50} values ranging between 70 and 19,300 nM), they are always more cytotoxic than the alkylating unit **16** alone (with only few exceptions). A fairly marked dependence on the number of pyrrolic rings for the antiproliferative activity has been observed in the **17a**–**c** series with compound **17c** comprising three pyrrolic rings proving to be the most active. The relationship between the number of pyrrolic groups in the **17d**–**f** series and their corresponding cytotoxicity did not seem to follow this pattern. In fact, the cytotoxicity was higher for the compound **17d**, which possesses only one pyrrolic ring. It is interesting to note that the L1210 cell line was 50-fold more susceptible to the cytotoxic action of compound **17f** than FM3A cells. For all cell lines, taken together, compounds possessing the same number of pyrrolic rings and the alkylating unit **15** appeared to be more cytotoxic than those containing **16** as the alkylating agent.

High-resolution denaturating gel electrophoresis indicated that **17c** selectively alkylates the third adenine of the 5′-ACAAAAATCG-3′ motif within a 400 bp DNA fragment, the strongest and most highly sequence-specific DNA alkylation activity observed. This compound elicited the strongest and most highly sequence-specific DNA alkylation activity. For compound **17c**, DNA alkylation was observed even at 50 nM. Results from this investigation suggest a promising approach for developing a new generation of DNA-alkylating agents based on CPI analogues and lexitropsin hybrid system that can alkylate purine bases in a sequence-selective fashion. Because of the high efficiency of alkylation, results from the present investigation suggest that these molecules should be useful in the design of compounds that target a single gene. Further studies on the generality and the optimization of this new class of DNA alkylation systems are currently in progress.

An approach, which has proved to be particularly successful, is represented by a novel series of hybrid molecules **18a**–**f**, namely a molecular combination of distamycin A and the antineoplastic agent uramustine. Uramustine (uracil mustard) **19** is an inexpensive oral alkylating agent that has been effective in the treatment of patients with lymphosarcoma,[34,35] chronic lymphatic leukemia,[36] and thrombocythemia.[37] Uramustine interacts in GC-rich regions being able to alkylate guanine-N7 in 5′-PyGCC-3′ (Py = pyrimidine) sequences.[38–40]

This homologous series **18a**–**f** consisted of distamycin A joined to uramustine by suitable aliphatic carboxylic acid moieties containing a flexible polymethylene chain, which is variable in length [$(CH_2)_n$, where $n = 1$ up to 6].[41] A flexible polymethylene spacer was chosen, allowing the nitrogen mustard of uramustine to interact more closely with the DNA target.



n=1, **18a**
n=2, **18b**
n=3, **18c**
n=4, **18d**
n=5, **18e**
n=6, **18f**

All the hybrid compounds in this series exhibit enhanced activity compared both to distamycin A and uramustine, giving IC_{50} values in the range 7.26–0.07 μM on K562 cells, with maximal activity shown when $n = 6$ (Table 3).

The distance between the uramustine and distamycin frame is crucial for the cytotoxicity, in fact as the size of the linker spacer increases from $n = 4$–6, the cytotoxic activity is also enhanced, with IC_{50} values in the range 0.07–0.14 μM. Compound **18f**, with the longest linker length in the series, was the most active compound with an IC_{50} value lower than that for distamycin. The higher cytotoxic activities of compounds **18d**–**f** could be also explained by an improved transportation of these molecules into the cells due to their increased lipophilicity.

DNase I footprinting experiments showed selective covalent binding of uramustine–distamycin hybrids **18a**–**f** to A+T-rich DNA sequences with a non-covalent binding specificity identical to that observed for distamycin. The compounds **18d**–**f** were more effective at producing footprints than **18a**–**c**. These corresponded to the sequences 5′-TTTTTG, AAAACG, and TTTTTA, respectively, with the alkylation on the last base (G/A).

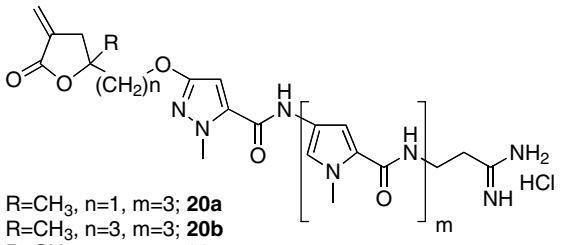
In the series of compounds **18a**–**f** progressive enhancement in cytotoxic potency with the length of the polymethylene chain does not correspond to increased alkylation intensity. Instead, this observation is tentatively attributed to enhanced cellular uptake due to increased lipophilicity as the polymethylene chain extends.

Table 3. In vitro activity of distamycin A, uramustine (**19**), and hybrid compounds **18a**–**f** against K562 human leukemia cell line

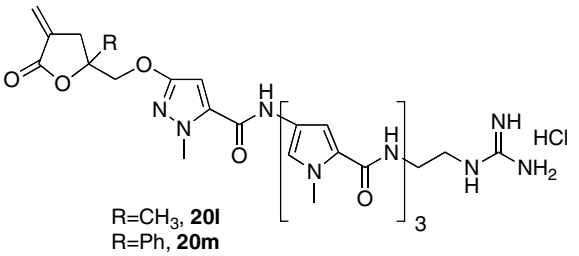
Compound	IC_{50} (μM ± SE)
Distamycin A	>100
19	5.1 ± 0.6
18a	4.06 ± 1.03
18b	2.54 ± 2.23
18c	7.26 ± 5.88
18d	0.11 ± 0.02
18e	0.14 ± 0.05
18f	0.07 ± 0

IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.

Another interesting class of hybrid conjugates **20a**–**m** were obtained tethering to a pyrazole derivative, which acts as a rigid linker, two different moieties. One is represented by a polypyrrole structurally related to distamycin A, to acquire DNA minor groove-binding activity, while the second moiety is an α-methylidene-γ-butyrolactone residue, with methyl, phenyl, and 4-substituted phenyl groups at the lactone C(γ) position.[42] α-Methylene-γ-butyrolactone derivatives have attracted much attention over the years since the α-methylene-γ-butyrolactone ring is an important functional structure in a wide range of natural products, particularly cytotoxic sesquiterpene lactones.[43,44] It was soon determined that the structural requirement for the biological activities is mainly associated with the exocyclic, conjugated double bond (the O=C=C=CH_2 moiety), which acts as an alkylating agent in a Michael-type reaction with biological cellular nucleophiles or sulfhydryl-containing enzymes.[45]



R=CH_3, n=1, m=3; **20a**
R=CH_3, n=3, m=3; **20b**
R=CH_3, n=5, m=3; **20c**
R=CH_3, n=7, m=3; **20d**
R=CH_3, n=1, m=2; **20e**
R=CH_3, n=1, m=1; **20f**
R=Ph, n=1, m=3; **20g**
R=Ph, n=1, m=2; **20h**
R=Ph, n=1, m=1; **20i**
R=p-Cl-C_6H_4, n=1, m=3; **20j**
R=p-C_6H_5-C_6H_4, n=1, m=3; **20k**



R=CH_3, **20l**
R=Ph, **20m**

For **20g**, **20h**, and **20m**, which possess the phenyl at the γ-position of the α-methylene-γ-butyrolactone moiety as alkylating moiety, the cytotoxicity of the hybrids was much greater than that of the α-methylene-γ-butyrolactone units tested alone (Table 4). The lower inhibitory potency of **20j** and **20k** with respect to **20g** implies that both an electron-donating substituent (Ph, compound **20k**) and an electron-withdrawing substituent (Cl, compound **20j**), at the benzene moiety of the lactone, reduced their antiproliferative activity.

Table 4. In vitro activity of hybrids **20a–m** against the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), and human T-lymphoblast (Molt/4 and CEM) cells

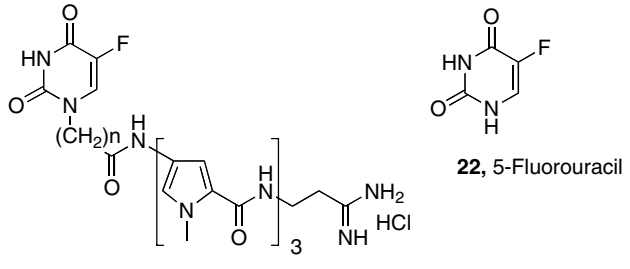
Compound	IC_{50} (μM)			
	L1210/0	FM3A/0	Molt4/C8	CEM/0
20a	14.7 ± 4.9	49.0 ± 3.7	14.7 ± 2.5	15.9 ± 1.2
20b	195 ± 12	243 ± 21	254 ± 11	255 ± 22
20c	109 ± 10	92 ± 16	106 ± 13	74.3 ± 3.8
20d	26.7 ± 1.1	63.3 ± 6.7	71.1 ± 2.2	35.6 ± 5.6
20e	8.02 ± 2.9	27.6 ± 11	6.8 ± 0.3	9.6 ± 4.7
20f	24.3 ± 4.0	32.4 ± 6.1	22.3 ± 10.1	26.3 ± 2.0
20g	4.2 ± 0.5	17.5 ± 1.2	5.2 ± 0.1	9.7 ± 4.2
20h	4.1 ± 0.1	18.5 ± 1.3	4.6 ± 0.4	5.7 ± 1.1
20i	12.5 ± 5.8	36.3 ± 20	15.8 ± 13	18.9 ± 3.2
20j	25.2 ± 2.4	64.7 ± 29	79.1 ± 31	86.3 ± 3.6
20k	33.2 ± 1.1	21.8 ± 9.2	65.3 ± 8.0	95.0 ± 0
20l	9.6 ± 4.5	42.5 ± 23	12.3 ± 7.7	15.9 ± 2.7
20m	5.1 ± 1.4	20.5 ± 4.8	10.1 ± 7.1	10.0 ± 1.1
Distamycin A (**1**)	133 ± 18	150 ± 29	64.6 ± 15	113 ± 26

IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.

Upon increasing the number of methylene units from one to three (compounds **20a** and **20b**, respectively), the cytostatic activity dramatically decreased. When the methylene linker length was increased from three (compound **20b**) to five (compound **20c**) methylene units, the in vitro antiproliferative activity increased at least 2-fold, but proved to be lower than that with $n = 1$ (derivative **20a**). Compound **20d** with the longest linker length in the series ($n = 7$) was more active with respect to the derivatives with a propyl and pentyl chain (compounds **20b** and **20c**, respectively), and comparable to compound (**20a**) with the shortest spacer ($n = 1$). In the series of derivatives **20a**, **20e**, and **20f**, which possess a α-methylene-γ-methyl-γ-butyrolactone moiety, the derivative **20e** with two *N*-methylpyrrolic units was almost 2-fold more cytostatic than the corresponding pyrrole homologue **20a**. The derivative **20a** showed an activity comparable to that of compound **20f** characterized by the presence of a single pyrrolic ring in its structure. The same behavior was not observed for the hybrids **20g–i**, characterized by the presence of a α-methylene-γ-phenyl-γ-butyrolactone moiety as alkylating unit. Compounds **20g** and **20h**, which possess three and two pyrrolic rings, showed a similarly pronounced antiproliferative activity and proved to be 3-fold higher than that observed for the monopyrrolic compound **20i.** The nature of the substituent at the C(γ)-position of the lactone moiety had a great effect on the antiproliferative activity of compounds having the same oligopeptidic frame. Comparing the cytostatic activity of the compounds with a different alkylating unit and with the same number of pyrrolic rings; the derivatives **20a**, **20e**, and **20f**, characterized by the presence of three, two, and one pyrrole units, and with the aliphatic methyl substituent at the γ-position of the lactone, were less active than their γ-phenyl counterparts **20g–i**, respectively. Modification of the C-terminal amidine group in compounds **20a** and **20g**, with a guanidino moiety to furnish the corresponding derivatives **20l** and **20m**, substantially maintained the cytostatic activity.

Using the human leukemia cell line HL-60, we have tested the effects of a selected series of compounds on programmed cell death (apoptosis). We found that **20g**, **20h**, and **20l**, but not **20e**, are able to induce apoptosis on HL-60 cells. Treatment of the tumor cells with these compounds induced morphological changes and DNA fragmentation characteristic of apoptotic cell death. Compound **20h** also induced extensive hydrolysis of poly(ADP-ribose) polymerase (PARP), considered to be a hallmark of apoptosis, which plays a critical role in chromatin architecture and DNA metabolism.

In some cases the hybrid drug approach apparently failed to present practical significant advantages in terms of activity, while in some cases these derivatives even lost the activity of the antitumor moiety, as occurred, for example, in a case concerning the tethering of anticancer drug 5-fluorouracil to distamycin A (compounds **21a–f**).[46] 5-Fluorouracil (5-FU, compound **22**) is an antimetabolite agent, used for the treatment of several malignancies (e.g. breast cancer, tumors of the gastrointestinal tract, and other solid tumors),[47] which expresses, after intracellular glycosylation, its antimetabolic potential by irreversible alkylation of thymidylate synthase (TS).[48]



22, 5-Fluorouracil

n=1, **21a**
n=2, **21b**
n=3, **21c**
n=4, **21d**
n=5, **21e**
n=6, **21f**

This homologous series **21a–f** consisted of distamycin A joined to 5-fluorouracil by aliphatic carboxylic acid moieties containing a polymethylene chain [$(CH_2)_n$, where $n = 1$ up to 6]. In these derivatives, 5-FU contains a C-6 electrophilic center, which can be positioned near nucleophilic centers on DNA, such as N3 of adenine in the minor groove or N7 of guanine in the major groove. A flexible polymethylene spacer was chosen, to allow 5-FU to accommodate closer to the DNA target. The general chemical reactivity of the 5-halogenated uracils accounts for a facile Michael-type conjugate addition at the C-6 pyrimidine ring. followed by a subsequent elimination of the C-5 halogen (Scheme 1).

The hybrids **21a–f** were tested in vitro against K562 cell line and compared to distamycin A. The derivative **21a**, which possesses the shorter spacer, is completely inactive. With respect to compounds **21b–f**, it is interesting to note that the modification in the length of the polymethylene spacer [$(CH_2)_n$, with $n = 2–6$] between distamycin and the 5-FU does not have major effects on the antitumor activity, that for the derivatives **21b–f** appear to be either less than or comparable with that of distamycin A (Table 5). Only compound **21b** shows a cytotoxic activity comparable to that of distamycin A, and increasing the length of the polymethylene chain from three to six does not induce large changes in the activity. In fact, the compounds **21c–f** proved to be less active with IC_{50} values of 82, 153, 50, and 50 μM, respectively. For these compounds the in vitro antitumor activity was not influenced by the length of the -$[CH_2]_n$ linker, being comparable to that of distamycin A. Arrested polymerase-chain experiments demonstrated selective binding of the 5-fluorouracil-distamycin hybrids to A+T-rich DNA sequences.

Another example of a failed hybrid drug approach was represented by the conjugates **23** and **24** constituted between the hydrophilic platinum (II) tethered to distamycin A by L-cysteine and D,L-2,3-diaminopropionic acid, respectively. *cis*-Diaminedichloroplatinum (II) (cisplatin, *cis*-DDP, **25**) is one of the major drugs in the treatment of a variety of human solid tumors such as testicular and ovarian tumors as well as head, neck, and lung tumors.[49] The primary mechanism of cytotoxicity of this drug is regarded as the consequence of making a covalent 1,2-intrastrand adduct with N7 of adjacent guanine residues in GC-rich sequences of DNA major groove, thus bending and modifying the DNA duplex.[50] Despite its success, the clinical usefulness of cisplatin is limited by its severe side effects such as dose-dependent nephrotoxicity, nausea and vomiting, ototoxicity, neurotoxicity, and myelosuppression. Some tumors have natural resistance to cisplatin, while others develop resistance after the initial treatment. A major component of resistance to cisplatin is reduced cellular uptake, ad it has been shown that increasing lipophilicity, and hence passive diffusion, can overcome the reduced uptake.[51] Cisplatin also has limited solubility in aqueous solution and it is administered intravenously.

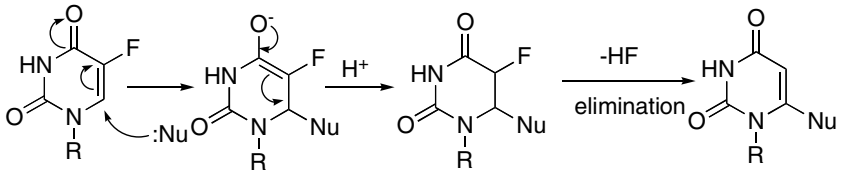
Table 5. In vitro antiproliferative activity of hybrids **21a–f** against K562 human leukemia cells

Compound	IC_{50} (μM ± SE)
Distamycin A	20
21a	>200
21b	23
21c	82
21d	153
21e	50
21f	50

IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.

A design strategy for the design of novel Pr drugs that may produce polyfunctional compounds with synergistic action includes the use of DNA minor groove binders as platinum carrier ligands, which could be able to target the Pt coordination moiety to DNA. Based on this strategy and due to the high affinity of distamycin A for the minor groove of DNA, we are expecting that these platinum complexes **23** and **24** would localize in the vicinity of the DNA, and the combined effect resulting from platination and minor groove binding might confer cytotoxic activity to these complexes.[52] Moreover, the presence of an amidino moiety in the distamycin frame should confer hydrophilicity to these platinum complexes, that may facilitate transport across the cellular membrane, increase both intracellular drug accumulation and interaction with the DNA, thus improving their effectiveness. In fact, it has been shown that increasing hydrophilicity can be correlated with increased activity.

Both the platinum complexes **23** and **24** proved to be completely inactive ($IC_{50} > 200$ μM) with respect to all the cell lines tested, and surprisingly the most interesting results were obtained when the related carriers **26** and **27**



21a-f

R= Distamycin A with an aliphatic carboxylic acid chain

Scheme 1.

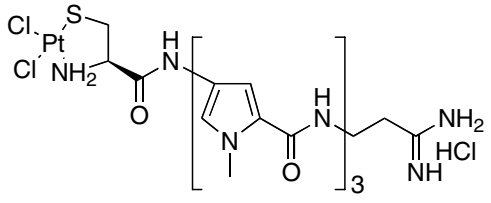
Table 6. In vitro activity of cisplatin (**25**), free ligands **26** and **27**, and platinum complexes **23** and **24** against Wil2-NS, CCRF-SB, RAJI, CCRF-CEM, MOLT-4, and K-562 leukemia cell lines

Compound	IC_{50} (μM)					
	Wil2-NS	CCRF-SB	RAJI	CCRF-CEM	MOLT-4	K562
23	>200	>200	>200	>200	>200	23
24	>200	>200	>200	>200	>200	20
26	98	92	45	36.7	35.8	24.2
27	108	97	42.8	40.6	38.8	21.7
Cisplatin (25)	12.8	11.9	11	3.7	4	5

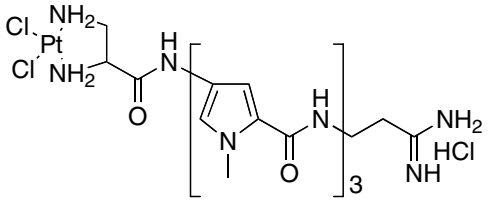
IC_{50}, 50% inhibitory concentration represents the mean from dose–response curves of at least three experiments.

were tested alone. In fact, as it is shown in Table 6 both the free ligands **26** and **27** were more active than the corresponding complexes **23** and **24**, with these former derivatives which were more active in this study against Raji, CCRF-CEM, and MOLT-4 with IC_{50} values ranging between 35 and 45 μM. However, it should be pointed out that **26** and **27** showed a cytotoxic activity much lower than that of reference compound cisplatin in all the cell lines tested. However, none of the synthesized compounds are as potent as cisplatin. The cytotoxic activity of compounds **23** and **24** underlines that there is not a combined effect of platination and DNA-binding activity, and that the ligands **26** and **27**, respectively, are not suitable carriers that favor DNA targeting by *cis*-Pt(II) centers.

The difference in activity between the two platinum complexes **23** and **24** with respect to the free ligands **26** and **27** could be due to many factors. The observed very small biological effect of **23** and **24** could be considered to a consequence of an insufficient penetrative activity of these platinum complexes across the cellular membrane and a consequent insufficient intracellular drug accumulation. It is possible that the high molecular weight of platinum complexes (MW > 800 Da) prevents them from passing through the cell membrane, whereas the



23



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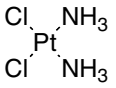
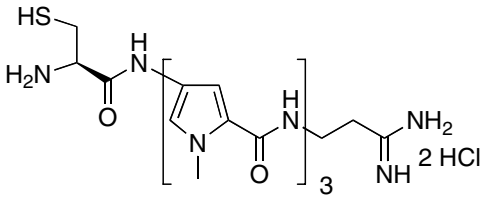
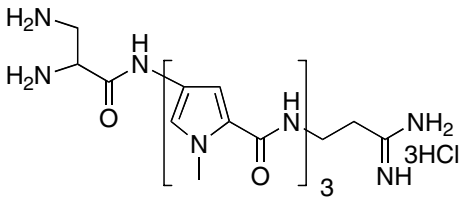


cis -platin (**25**)



26



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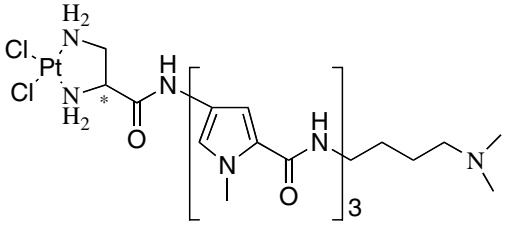


28 (R and S)

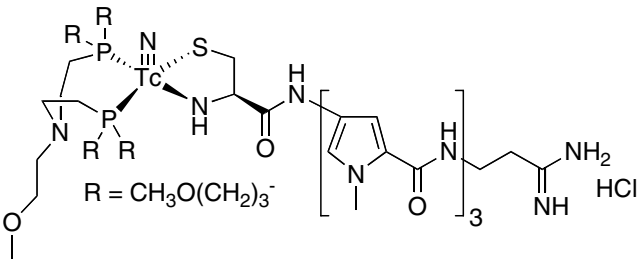
reduced molecular weight of ligands **26** and **27** could allow them to pass more easily into the cell.

Another factor is the water solubility. Unfortunately, the platinum complexes **23** and **24** are substantially less water-soluble than cisplatin. The water solubility of these derivatives may be increased replacing the chloride ligands (which act as leaving groups) with chelating carboxylates (such as cyclobutane dicarboxylate), oxalate, and glycolate.

By the synthesis of the (*R*) and (*S*) isomers of compound **28**, where the *N*-propionamidine terminus of **24** was replaced with a *N*,*N*-dimethybutylamino moiety, Brabec showed that the attachment of distamycin to cisplatin mainly affects the sites involved in the interstrand cross-links so that these adducts are preferentially formed between complementary guanine and cytosine residues. This interstrand cross-link bends the helix axis by 35° toward minor groove, unwinds DNA by approximately 95°, and distorts DNA symmetrically around the adduct. This distortion was similar for both isomers, symmetrical around the cross-linked base pair and extended mainly over five base pair.[53]

Recent work of failed hybrid approach was represented by the synthesis of compound **29**, in which a simplified derivative of the DNA cross-linking agent isochrysohermidin **30**[54] was tethered to distamycin A derivatives with a *N*,*N*-dimethylpropylamine which replaced the amidine side chain.[55] Compound **29** showed a very low antiproliferative activity (IC_{50} superior to 200 μM) against L1210 cell line and it exhibited no time-dependent increase in DNA-binding affinity, indicative of a slow, reversible covalent attachment to DNA.

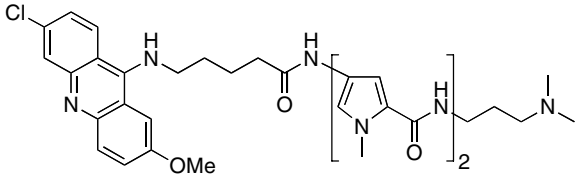
Radiopharmaceuticals are radiolabeled substances containing a radioisotope, and designed to target specific sites of disease such us neoplastic tissues. In the last decades, there has been a growing interest in developing radiolabeled compounds for treatment of metastatic cancer.[56] Our group has reported the first example of a hybrid constituted by distamycin A and cysteine labeled with the γ-emitting radionuclide ^{99m}Tc to afford the conjugate complex **31**, which appears to be sufficiently stable for in vivo applications as tumor imaging agent using single photon emission tomography (SPET).[57] This new radiopharmaceutical is of potential interest as tumor imaging agent in diagnostic nuclear medicine. This technique utilizes the emission characteristic of certain γ-emitting radioisotopes, which are incorporated into radiopharmaceuticals having the appropriate biodistribution properties to accumulate in selected target tissues.



31

Complex **31** was found to be stable both in physiological solution and in plasma, by measuring its radiochemical purity (RCP) by TLC chromatography over a period of six hours. No significant alteration of RCP was observed under these conditions. This is an essential requirement for a radiopharmaceutical being used as diagnostic or therapeutic agent.[58] Further studies, carried out both in vitro and in vivo using animal models, are underway to establish the ability of complex **31** to target specific tumor cell lines. Due to the chemical similarities between technetium and rhenium, complex **31** may be viewed as a suitable model for the design of ^{99m}Tc- and $^{186/188}$Re-radiopharmaceuticals potentially useful for the diagnosis and treatment of various types of cancerous diseases.

As a method to approach a new chemotherapy to treat cancer more effectively and to reduce side effects, Kawanishi has demonstrated that DNA cleavage induced by antitumor drug C1027 was enhanced through intermolecular reaction with DNA by quinacrine–netropsin hybrid molecule **32** as DNA-binding ligands.[59]



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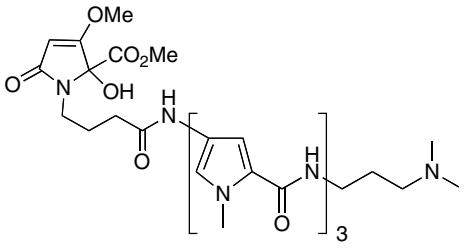

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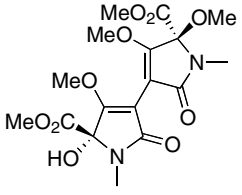


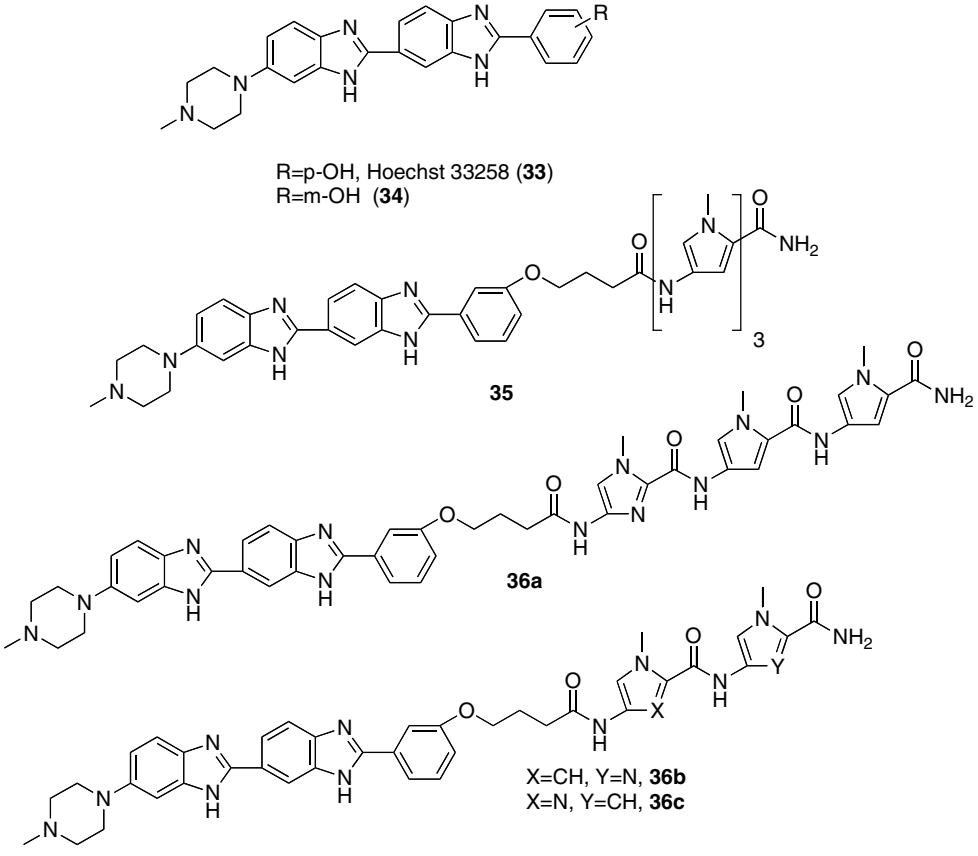
isochrysohermidin **30**

In search for potential minor groove binders targeting longer sequences, an interesting hybrid molecule was obtained combining an analogue of synthetic bis-benzimidazole dye Hoechst 33258 (also known as Pibenzimol, **33**) with a tripyrrole polyamide structurally related to distamycin A with a γ-hydroxybutyric acid linker. Hoechst 33258 is AT-selective for DNA and binds in the minor groove of B-DNA[60] where it offers a protection pattern against DNA cleavage by footprinting agents that is essentially similar to those by netropsin and distamycin, suggesting a binding size to four or five consecutive AT-base pairs. The Hoechst 33258–DNA interaction appears to be stabilized by a pair of bifurcated hydrogen bonds and van der Waals contacts, but in fact these molecular forces are believed to contribute little to overall binding affinity.[61] The analogue **34** of Hoechst 33258, bearing a phenolic hydroxyl group in the meta rather than para position, was considerably less cytotoxic than the para one[62] and this difference may reflect ligand access to the nucleus once internalized. The tripyrrole-Hoechst conjugate **35** binds to nine-bp long A/T-rich sites at subnanomolar concentrations.[63]

On compound **35**, the replacement of one pyrrolic ring with an imidazolic moiety furnished a series of compounds with structure **36a**–**c**. Conjugate **36a** recognizes the nine-bp site, and it is highly selective for the G/C containing nine-bp site 5′-gcggTATGAAATTcgacg-3′. Substitution of one of the Py moieties with Im, conjugates **36b** and **36c**, allows recognition of G/C in the seven-bp site. Conjugates **36b** and **36c** are specific for 5′-gcggtaTGAAATTcgacg-3′ and 5′-gcggtaCAAAAT Tcgacg-3′ sites, respectively.[64]

Among the topoisomerase I inhibitors, Bailly has reported a series of potential DNA minor groove binder inhibitors of topoisomerases characterized by 4-arylcarboxamido-pyrrolo-2-carboxamido skeleton which combines the structural features of distamycin A and furamidine. The amidine side chain was replaced with more stable primary amine-containing chains. The central pyrrole ring was substituted with either a *N*-methyl group as found in distamycin or with a more bulky *N*-benzyl group. The three compounds synthesized, with formula **37a**–**c**, showed apparently no correlation between the IC_{50} values and their effects on topoisomerase I, but interestingly a link can be established with the DNA-binding data. The fact that the *N*-benzyl derivative **37c** is about 4 times less cytotoxic than compound **37a** likely accounts for its reduced capacity to interact with DNA. Otherwise, the derivatives bearing a *N*-methyl (**37a**) or *N*-benzyl pyrrole (**37b**) stabilize topoisomerase I–DNA complexes.[65] The *N*-methyl pyrrole ring was employed for the synthesis of a new class of molecules with low topoisomerase I inhibitor (5–50 μM), where the compound **38** became the most active compound of the whole series.[66]

Linking a DNA minor groove-binding ligand to the NH_2 or CH_2OH function of the DNA topoisomerase



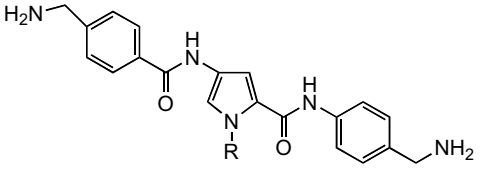
II inhibitor 9-anilino-acridine (AHMA) derivative, have been synthesized two series of derivatives with general formula **39a–d** and **40a–d**, respectively. The in vitro antitumor activities on a variety of human tumor cell lines revealed that derivatives **40a–d**, linked with one or two pyrrole units to the CH_2OH function of AHMA, were more cytotoxic than **39a–d**. In the same study, all compounds bearing one pyrrole ring were more potent than those compounds that possess two pyrrole rings. Moreover, derivatives with general formula **40a–d**, bearing a succinyl chain as the linker spacer, were more potent than those compounds having a glutaryl bridge. Among these hybrid molecules, the compound **40a** was 2- to 6-fold more cytotoxic than the parent compound AHMA.[67]

3. Hairpin pyrrole-imidazole polyamide conjugates

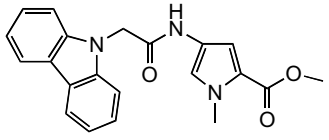
Once distamycin and its conjugates were found to bind as a dimer in the minor groove, polyamides containing the equivalents of a dimer structure were constructed. The hairpin pyrrole-imidazole, constituted by 6- or 8-pyrrole-imidazole polyamide with a linker region (usual a γ-aminobutyric acid) at the center of the molecule, is the prototype of an artificial organic molecule that can bind to the DNA minor groove with a highly sequence-specific manner and dissociation constants in the nanomolar range.[68–70] The presence of a β-alanine or a (3-dimethylaminopropyl)amino group at the C-terminus facilitates recognition of AT residues. The synthetic polyamides exploit 2:1 binding mode and recognize specific sequences by the side-by-side pairing of aromatic amino acids in the minor groove. Pyrrole opposite imidazole (Py/Im) targets a C–G base pair, whereas Im/Py targets G–C. The Py/Py pair binds both A–T and T–A base pairs.[71]

A recent example of this class of compounds is the hairpin polyamide Im-Py-Py-Py-$(R)_{H2N}\gamma$-Im-Py-Py-Py-β-Dp (**41**), which is able to recognize the binding site (5′-WGWWCW; W=A or T). DNase I footprinting confirmed that **41** binds to the sequence AGAACA at nanomolar concentrations and that changing the terminal A to G causes a dramatic decrease in affinity, while there was no interaction with the reverse sequence WCWWGW. Fluorescence melting studies with 11-mer duplexes showed that the polyamide had very different effects on the forward (TGWWCT) and reverse (TCTAGT) sequences. At low concentrations, the polyamide produced biphasic-melting curves with TGATCT, TGTACT, and TGAACT, suggesting a strong interaction.[72]

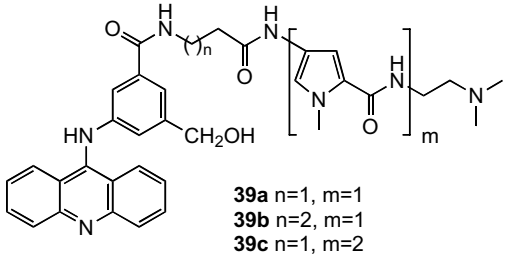
The potential of hairpin polyamides as the gene regulator was previously reviewed.[73,74] Several structural modifications on the structure of hairpin compounds have been reported in order to modify the functions and properties. The eight-ring hairpin polyamide **42** containing *N*-methylimidazole and *N*-methyl pyrrole amino acids, with a second positive charge at the C terminus, showed activity against a number of clinically relevant fungal strains in vitro, and activity against *Candida albicans* in a mouse model. Increasing G–C content within the hairpin target site decreases antifungal activity. Experiments performed with a panel of mutant strains indicate that a discrete number of genes are effected by this compound. These results indicate a potential DNA-binding mechanism of action for the antifungal activity, demonstrating that **42** may have potential as novel mechanism of action for antifungal agents.[75]



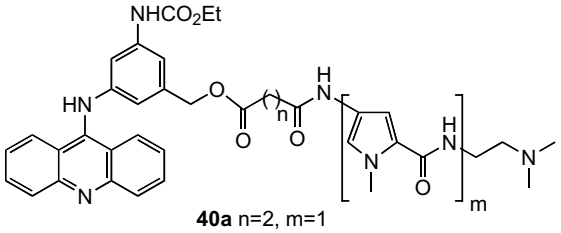
37a, R=H
37b, R=Me
37c, R= Bn



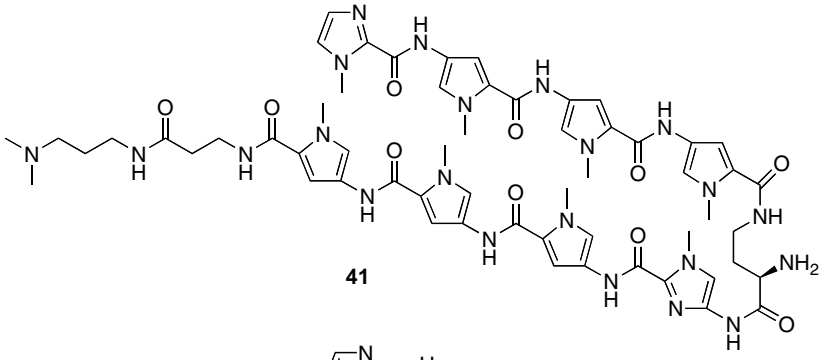
38



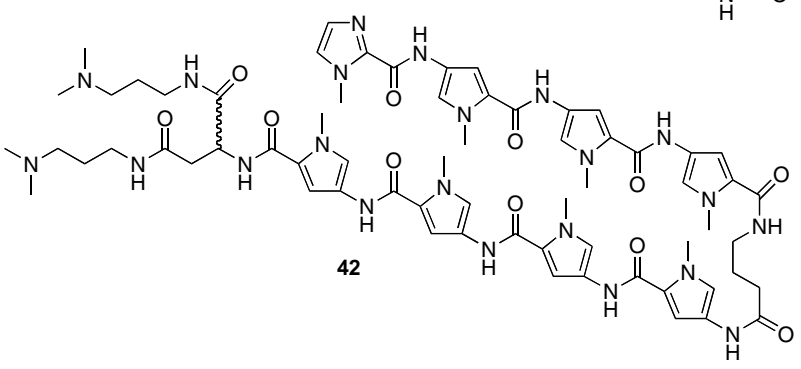
39a n=1, m=1
39b n=2, m=1
39c n=1, m=2
39d n=2, m=2



40a n=2, m=1
40b n=3, m=1
40c n=2, m=2
40d n=3, m=2

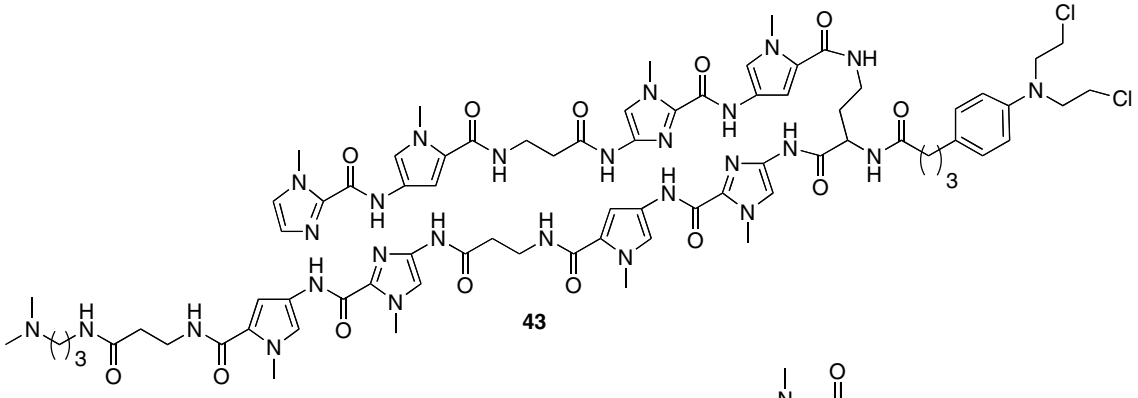


41

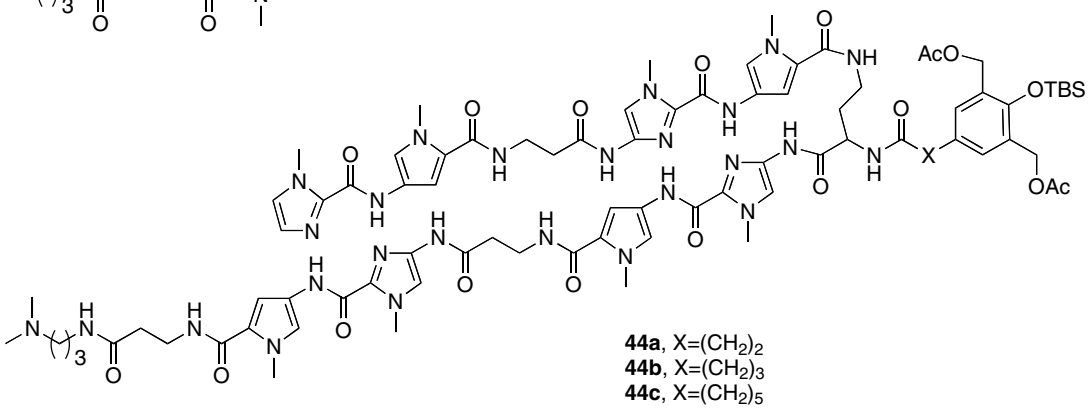


42

Compound **43** represented an interesting prototype of a novel class of DNA alkylating agents, which combines the DNA cross-linking moiety chlorambucil (CHL) with a sequence-selective hairpin pyrrole (Py)-imidazole (Im) polyamide (ImPy-β-ImPy-γ-ImPy-β-Dp). The conjugate **43** alkylates predominantly at 5′-AGCTGCA-3′ sequence, which represents the polyamide-binding site and it was able to inhibit the growth of Jurkat and 293 cell line with IC_{50} values of 2.2 and 13 nM, respectively.[76] The hairpin ImPy-β-ImPy-γ-ImPy-β-Dp was also conjugated with a series of quinone methide precursors designed for DNA cross-linking to the minor groove (compounds **44a**–**d**). Although reaction was only observed for DNA containing the predicted recognition sequence, yields of strand alkylation were low. Interstrand cross-linking was more efficient than alkylation but still quite modest and equivalent to that generated by the conjugate containing the *N*-mustard chlorambucil (compound **43**).



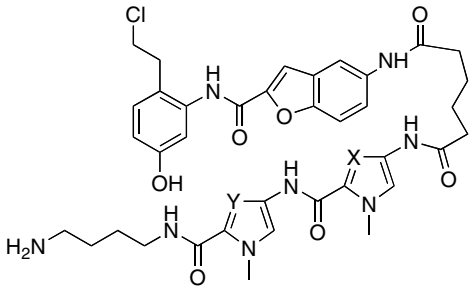
43



44a, X=$(CH_2)_2$
44b, X=$(CH_2)_3$
44c, X=$(CH_2)_5$
44d, X: CH=CH

Varying the length of the linker connecting the polyamide and quinone methide derivative did not greatly affect the yield of DNA cross-linking. Instead, intramolecular trapping of the quinone methide intermediate by nucleophiles of the attached polyamide appears to be the major determinant that limits its reaction with DNA.[77]

Further hairpin conjugates of achiral seco-cyclopropaneindoline-2-benzofurancarboxamide (achiral seco-CI-Bf) and three diamides (ImPy **45a**, PyIm **45b**, and PyPy **45c**), linked by a g-aminobutyrate group, provided additional efficiency and selectivity for alkylating specific sequence of DNA. The results provide evidence that hairpin conjugates of achiral seco-CI-Bf-polyamides could be tailored to target specific DNA sequences according to a set of general rules: the achiral CI moiety selectively reacts with adenine-N3, a stacked pair of imidazole/benzofuran prefers a G/C base pair, and a pyrrole/benzofuran prefers an A/T or T/A base pair.[78]



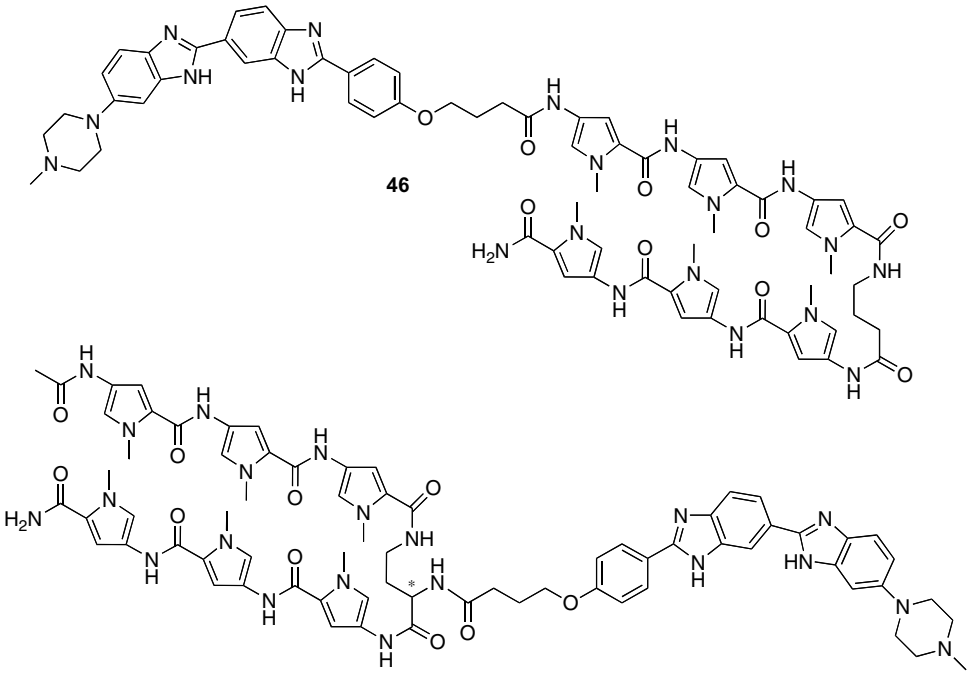
45a, X=N, Y=CH
45b, X=CH, Y=N
45c, X=Y=CH

Based on the sequence specificity and high affinity of polyamides for the minor groove, the conjugation with a bis-benzimidazole Hoechst 33258 analogue, to furnish the hybrid compound PyPyPy-γ-PyPyPy-γ-Ht (**46**), greatly enhances cellular uptake while maintaining sequence specificity.[79] The polyamide of the conjugate was observed to bind in a hairpin motif forming 1:1 conjugate–DNA complexes. The conjugate is able to recognize nine contiguous A/T bps, discriminating from the sequences containing fewer than nine contiguous A/T bps.

The Hoechst 33258 analogue was also linked at the α-position of the chiral amino acid incorporated in hairpin polyamides, to furnish the enantiomeric conjugates **47a** and **47b**. Both these derivatives recognize a 10 bp sequence. Interestingly, R-enantiomer **47a** exhibited 10- to 30-fold higher binding affinities than S-enantiomer **47b** for the DNA sequences studied. These binding differences were accounted for by molecular modeling studies, which revealed that the amide proton nearest to the chiral center in R-conjugate **47a** is better positioned to form hydrogen bonds to the DNA bases, while S-conjugate **47b** does not.[80]

The potential of hairpin polyamide as a new tool for electrochemical gene detection technology was achieved by the preparation of the minor groove binder **48** having a redox active ferrocene dicarboxamide moiety as linker.[81,82]

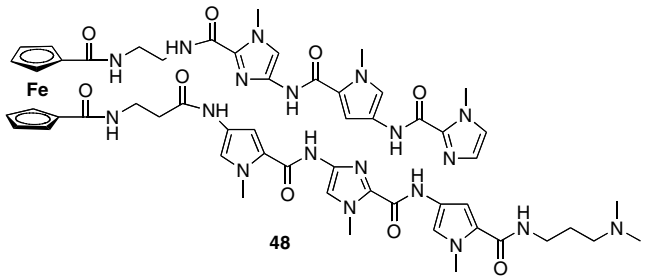
This derivative is the first example of the development of DNA minor groove binders by use of the structural properties of ferrocene. By the comparison between the minor groove width and the molecular



46

47a, R isomer
47b, S isomer

size of ferrocene, the insertion of a ferrocene moiety did not interfere with the binding of the minor groove binder to target DNA duplex. Therefore, it must be possible to design new minor groove binders by the combination of various heteroaromatic compounds, other than pyrrole-imidazole amides, and ferrocene-type scaffolds.



48

Another interesting class of head-to-head-linked hairpin oligo (*N*-methylpyrrole) carboxamides was constituted by compounds **49a–d**, in which deprotection and functionalization of the primary amino group with chemically or photochemically active moieties will permit building of highly specific synthetic DNA-directed enzyme-like molecules. Replacement of one or several *N*-methylpyrroles by *N*-methylimidazoles or other specific monomers 2,6 could make it possible to construct asymmetrical bis-conjugates that would be able to recognize mixed A/T:G/C sequences.[83]

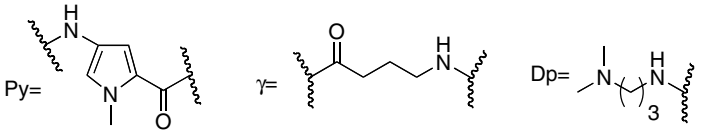
Their ability to bind double-stranded DNA and sequence specificity were compared and the apparent K_d values of their DNA complexes were determined. These compounds, particularly those with iminodiacetic linkers, revealed a high affinity for DNA (K_d = 4.5–4.8 × 10^{-9} M) and sequence-specific recognition of 9–10 base pairs.[6]

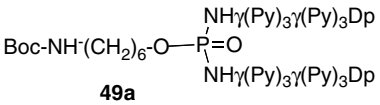
4. Conclusions

Hybrid compounds in which known antitumor have been tethered to distamycin frame are still an interesting class of DNA ligands which might have a therapeutic role in cancer. The present work demonstrated the validity of hybrids approach between distamycin A and derivatives of naturally occurring antitumor antibiotics with DNA-alkylating properties. Several of these hybrid derivatives show promising activity, which in some cases resulted much greater activity than that of the alkylating units alone. The hybrid approach with the nitrogen mustard derivative uramustine has proved so far to be particularly advantagious in terms of activity and represents an important model for the design of new cytotoxic minor groove binders, having disclosed the possibility of obtaining potent agents by combining moieties of mild cytotoxic activity with a DNA-binding frame derived from distamycin A, acting as a sequence-selective vector. In several cases hybrid drug approach apparently failed to present practical significant advantages in terms of activity, while in some cases these derivatives even lost the activity of the antitumor moiety, as occurred for example in the case concerning the tethering of DNA cross-linking agent isochrysohermidin to a distamycin-like frame.

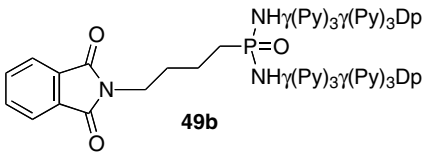
The fact that distamycin can inhibit binding to G4 DNA by a specific conserved polypeptide sequence suggests that new distamycin derivatives might be useful in the design of therapeutics targeted against specific proteins in vivo. Overall, the results of the study on compound C could represent a useful starting point for the synthesis of more potent telomerase inhibitors.

Finally, a better cellular uptake and a subsequent nuclear localization of hairpin polyamides will increase the potential utility of this class of molecules as selective inhibitors of target genes on cancer cells.

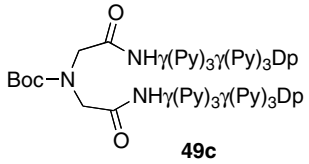




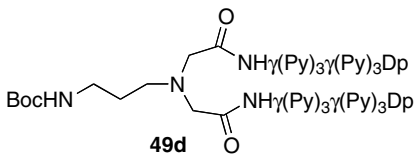
49a



49b



49c



49d

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