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DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF A PYRROLO [2,1-c][1,4]BENZODIAZEPINE (PBD)-DISTAMYCIN HYBRID

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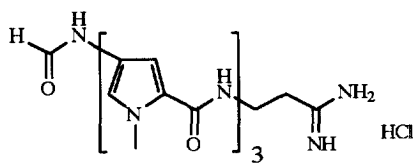
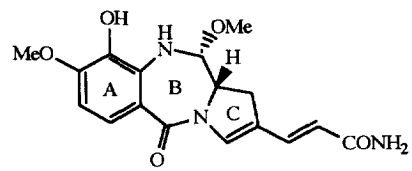
Abstract: We report the synthesis of a new hybrid **13** which is a combination of the naturally occurring antitumor agent distamycin A **1** and the pyrrolo[2,1-c][1,4]benzodiazepine **11**, related to the naturally occurring anthramycin **2**. The antitumor activity of the hybrid **13** was tested *in vitro* and compared to the natural product distamycin **1** and the PBD **11.**

Introduction

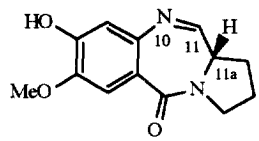
In the last few years, a growing interest has been shown in the development of DNA minor-groove binders acting as vehicles for the delivery of alkylating agents to DNA targets, and typical examples of this new class of compounds include alkylating derivatives of distamycin A **1**[1]. Distamycin A is a naturally occurring antiviral agent, isolated from cultures of *Streptomyces distallicus* [2,3]. Distamycin displays a high affinity for AT-rich sequences and binds reversibly, by noncovalent interactions, to the minor groove of double-helical B DNA. The pyrrolo[2,1-c][1,4]benzodiazepine (PBD) group[4], which includes the natural compounds anthramycin **2**[5] and DC-81 **3**, owes its DNA-interactive ability and resultant biological effects to a 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 adducts have shown a high sequence-specificity, for G-C rich DNA regions, in particular for X-G-X triplets (X=purine)[6].



1 Distamycin A



2 Anthramycin



3 DC-81

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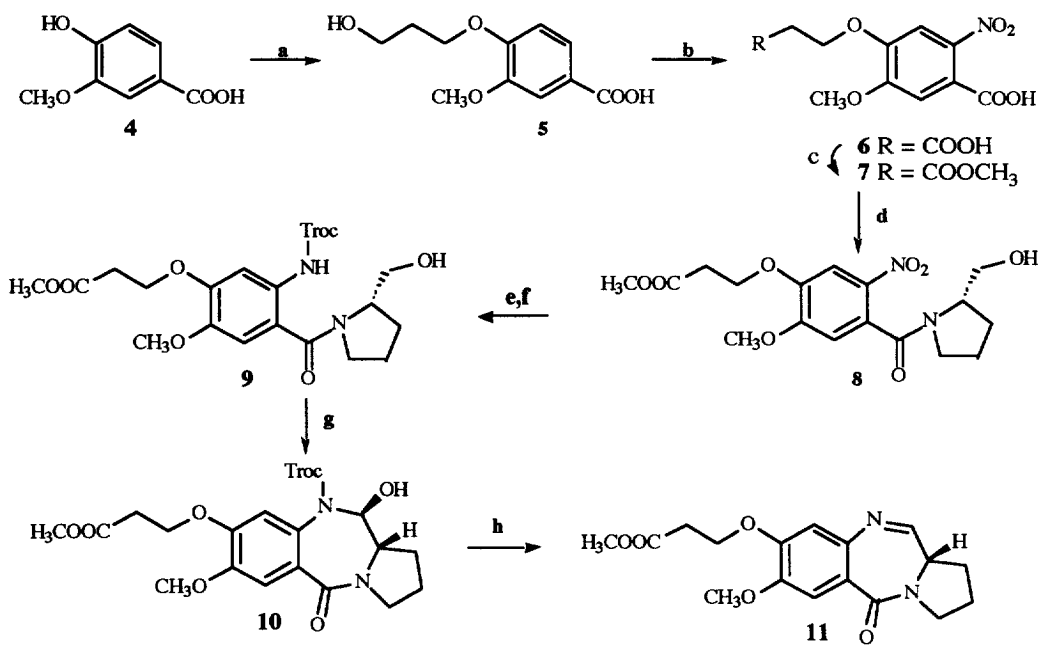
PII: S0960-894X(98)00544-7

Both distamycin A and the PBDs represent models for the preparation of new derivatives, which could be much more cytotoxic than the parent compounds. For this reason we have synthesized a hybrid compound between a new PBD **11**, able to bind covalently to GC-rich sequences, and distamycin which possesses a different base specificity.

Chemistry

The synthesis of the DC-81 related PBD **10** has been carried out by modification of a new general strategic route for PBD synthesis involving the use of N10-Troc-protected intermediates[7]. The reaction of vanillic acid **4** with 3-bromopropanol gave the compound **5**. Nitration of **5** with nitric acid and simultaneous oxidation of the alcoholic function afforded the nitro acid **6** which by chemoselective esterification in presence of *p*-toluenesulphonic acid yielded the nitro monoester **7** (61% yield, 3 steps). Coupling with 2 *(S)*-(+) pyrrolidinemethanol (*via* the acid chloride), to give **8**, followed by catalytic hydrogenation (Pd on C) of the nitro group and protection of the newly formed amino functionality with 2,2,2-trichloroethylchloroformate (Troc-Cl)[8] achieved compound **9** (50% yield, 4 steps) without any proof of racemisation at C11a. Swern oxidation of **9** gave the N10-Troc protected PBD **10** (82%) which was converted to **11** by cleavage of the Troc group with Cd/Pb couple in aqueous NH_4Ac[7,9] (Scheme 1).

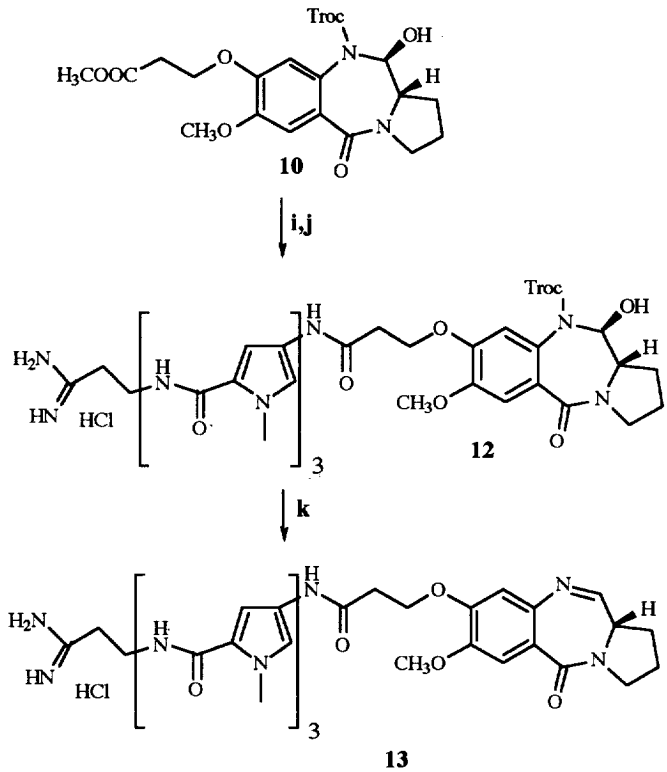
Scheme 1



Reagents: a: 3-bromopropanol, aq. NaOH, reflux; **b:** HNO_3 70%; **c:** CH_3OH, Tosyl-Cl (0.1 eq); **d:** i) $(COCl)_2$, dry CH_2Cl_2; ii) 2 *(S)*-(+) pyrrolidinemethanol, Et_3N, dry CH_2Cl_2; **e:** H_2/Pd(C), CH_3OH; **f:** Troc-Cl, pyridine, dry CH_2Cl_2; **g:** Swern oxidation; **h:** 10% Cd/Pb couple, CH_3OH, 1M NH_4Ac.

Acid hydrolysis of the ester **10** followed by coupling with deformyl distamycin[10] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) as condensing agent, yielded the Troc-protected hybrid molecule **12** purified by flash chromatography. The deprotection of the Troc group with the previously described methodology[9], afforded the free N10-C11 imine moiety of the target molecule **13**[11], which could be easily stored as the stable hydrochloride salt after purification by preparative HPLC and immediate lyophilization (Scheme 2).

Scheme 2



Reagents: i: THF, HCl 10%; **j:** deformyl distamycin (1 eq.), EDCI, DPEA, dry DMF; **k:** 10% Cd/Pb couple, CH_3OH, 1M NH_4Ac.

Results and Discussion

The activity of synthesized compounds **11**, **13** and distamycin A **1** on *in vitro* cell proliferation was studied on the human leukemia K562 cell line[12]. In order to study sequence-selective effects of the DNA-binding drugs a previously described polymerase chain reaction (PCR)[13] approach was employed. The human oestrogen receptor (ER) gene[14] and the Ha-ras oncogene[15] were chosen as model systems, suitable for determining sequence-selective binding of DNA-binding compounds when amplified by PCR. We recently published the nucleotide sequence of a 3.2 Kb genomic region located upstream of the oestrogen receptor sequence originally designated exon[16], and demonstrated that this region contains A+T

rich sequences recognized by distamycin A and distamycin analogues[17,18]. Ha-ras oncogene sequences, on the other hand, contain G+C rich regions and therefore interact with a low efficiency with distamycin, being on the contrary efficiently recognized by G+C selective binders, such as chromomycin and mithramycin[19]. The A+T/G+C ratio of the oestrogen receptor product is 3.46, while the A+T/G+C ratios of Ha-ras-1 and HIV-1 LTR PCR products are 0.6 and 0.67, respectively. We have previously demonstrated that A+T selective DNA-binding drugs such as distamycin and U-71184 inhibit generation of ER PCR products, being ineffective with Ha-ras PCR[12]. Conversely, G+C selective DNA-binding drugs, such as mithramycin and chomomycin, inhibit Ha-ras PCR products without affecting ER PCR[13,18-20].

In Table I we have reported, as a summary of three independent experiments, the effects of distamycin, **11** and **13** on *in vitro* cell proliferation (cell growth) of K562 cells (left column), showing that **13** retains a higher antiproliferative activity with respect to **11** and distamycin.

Table I

Compound	Inhibition of Cell growth[21] in K562 IC_{50} (μM)	Polymerase-chain reaction IC_{50} (μM)	
		ER[22]	Ha-ras[23]
Distamycin A	5	50	>200
11	1	>300	>300
Distamycin A+ 11	1	50	>200
13	0.2	8	8

(IC_{50}) = Inhibitory concentration (μM) necessary to obtain 50% inhibition of cell growth or generation of PCR products.

IC_{50} was found to be 5 μM for distamycin and 1 μM for **11**. No additive effects were observed when the two DNA-binding agents distamycin and **11** were added together (IC_{50} = 1 μM). On the contrary IC_{50} was found to be 0.2 μM for the hybrid **13**. Accordingly, the combined use of distamycin plus **11** suppress K562 cell growth only at 2.5 μM, while 0.5 μM final concentration of the hybrid **13** was found to be sufficient (data not shown). Since a possible explanation for this effect is a higher DNA-binding affinity for **13**, polymerase-chain reaction experiments were performed[24] (Table I, right column).

As already published by Gambari's group[20], distamycin inhibits ER PCR but is ineffective in inhibiting Ha-ras PCR, thus confirming a selective binding of distamycin to A+T rich gene sequences. By contrast **11** is not an effective inhibitor of the polymerase-chain reaction, confirming the previously published results showing that the kinetics of binding of pyrrolobenzodiazepines to DNA are very slow[25,26]. Accordingly, a combined treatment of the PCR mixture with **11** plus distamycin inhibits only the generation of ER PCR products. IC_{50} is also in this case 50 μM, identical to that found for distamycin

alone. This finding does not support any additive effects of **11** and distamycin. When **13** was added to PCR mixture, two clear effects were observed. First, this compound inhibits the generation of PCR products (IC_{50} = 8 μM) with high efficiency. Second, sequence-selective binding typical of distamycin is completely lost, as **13** inhibits also the Ha-ras PCR. These results were confirmed by DNase I footprinting experiments (data not shown), and strongly suggested that **13** is much more active that **11** or distamycin in binding to DNA. However, unlike distamycin, **13** binds also to G+C rich DNA sequences.

In summary, *in vitro* studies on cell growth and arrested PCR clearly demonstrate that the hybrid **13** is much more active than distamycin and **11** in (a) inhibiting cell proliferation of neoplastic cell lines and (b) binding to DNA sequences. As expected the DNA-binding selectivity of **13** is different than that of distamycin, being **13** capable to bind also to G+C rich elements.

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11. Compound **13**: Off white solid; m.p. (uncorrected): 263-264°C (dec.); $[\alpha]_D^{25}$(CH_3OH): - 11.2 (c= 0.16). ^1H NMR (DMSO-d_6) δ: 1.9 (m, 4H); 2.6 (t, J= 6.4 Hz, 2H); 2.78 (m, 2H); 3.4 (m, 4H); 3.73 (s, 3H); 3.77 (s, 3H); 3.80 (s, 3H); 3.84 (s, 3H); 4.3 (m, 2H); 5.7 (s, 1H); 6.9-7.3 (m, 8H); 8.2 (t, J = 7.3 Hz, 2H); 8.68 (s, 2H); 9.01 (s, 2H); 9.94 (s, 1H); 9.95 (s, 1H); 10.1 (s, 1H). Elemental analysis (calcd): C 56.23; H 5.61; N 19.5; Cl 4.49; (found): C 55.99; H 5.53; N 19.7; Cl 4.31. Mass (Maldi-Tof): 756 (M+2).
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21. IC_{50} was determined by comparing the cell number/ml of K562 cells treated with DNA-binding drugs to the value of untreated control cell cultures after 48h of continuos exposure to at least 4 concentrations of each drug.
22. For the amplification of the 5'-region of the human oestrogen receptor gene, the target DNA was either the pBLCAT8ERCAT1 plasmid or human genomic DNA.
23. The amplification primers for codon 61 regions of the Ha-ras oncogene were from the ras PointPrimers™ kit (Oncogene Sciences, Uniondale, NY, USA). For the amplification of the Ha-ras oncogene, the target DNA was human genomic DNA.
24. The effects of DNA-binding drugs were analysed after incubating target DNA at room temperature, for 5 min, with increasing amounts of the compounds, as reported in the text, followed by polymerase chain reaction. Amplified DNA was analysed by electrophoresis on 2.5% agarose, in TAE (0.04 M Tris-acetate, 0.001 M EDTA), 0.5 μg/ml ethidium bromide. Conditions of Ha-ras PCR were: denaturation, 92 °C, 45 sec; annealing, 62 °C, 45 sec; elongation, 72 °C, 30 sec (32 cycles); conditions of oestrogen receptor PCR were: denaturation, 92 °C, 45 sec; annealing, 58 °C, 1 min; elongation, 72 °C, 1 min (32 cycles).
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