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Synthesis and DNA Binding Properties of Amide Bond-Modified Analogues Related to Distamycin

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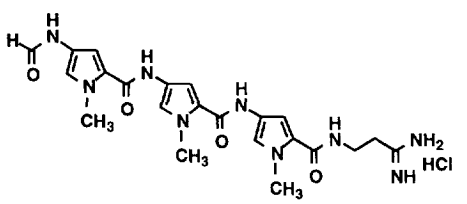
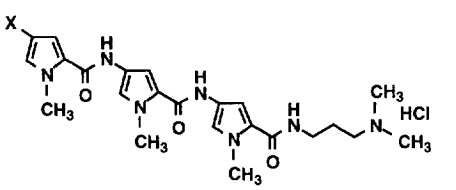
Abstract : Novel nitro analogues of distamycin which have *trans* olefin or α-diketo moiety instead of amide bond were synthesized. Their DNA binding properties studied by ethidium displacement assay and MPE footprinting experiments were also described. Copyright © 1996 Elsevier Science Ltd

There is considerable interest in the development of agents which recognize and bind to DNA in a sequence specific manner. Interest is particularly focused on the minor groove binding agents. The naturally occurring tripeptide antibiotic distamycin (**1**) originally isolated from *Streptomyces Distallicus* is a nonintercalative minor groove binder with high selectivity for AT-rich sequences[1, 2]. Recently several derivatives of **1** have been prepared and examined for their biological activities including DNA binding properties[3]. Among the structural modifications, it has been shown that replacement of pyrrole rings with other heterocyclic moieties (e.g. imidazole or thiazole) creates compounds with increased tolerance for GC base pairs at their binding sites[4].

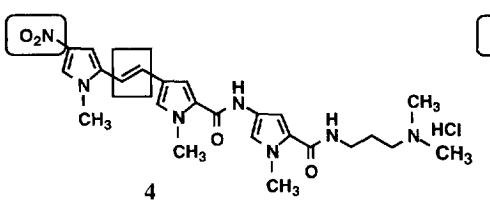
Scheme 1



Distamycin (**1**)



2 X = HCONH-
3 X = NO_2-



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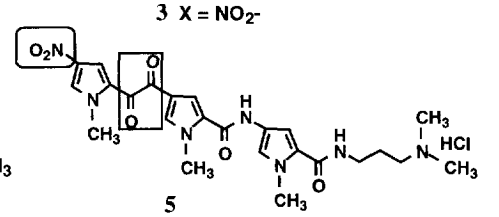

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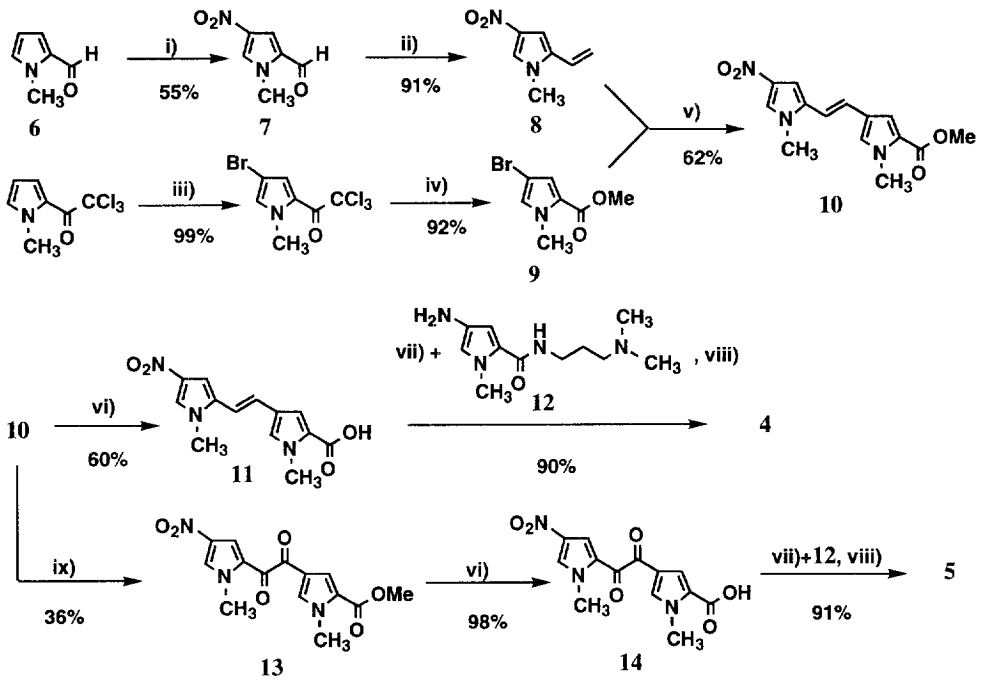
As shown in Scheme 1, we focus on the variation of the N-terminal formamide and the interpyrrole amide function of **1**. Herein we report the synthesis of novel nitro derivatives of distamycin which have

trans-olefin or α-diketo interpyrrole connection instead of amide and also report their DNA binding properties. The compounds **2** and **3**, which have formamide and nitro function in the N-terminal respectively, were prepared according to the known procedure[3b, 5]. Synthesis of novel nitro analogues **4** and **5** which one of the interpyrrole linkage is replaced by *trans*-olefin and α-diketone respectively are shown in Scheme 2. In every case dimethylamino group was introduced in the C-terminal of the analogues instead of the amidine present in **1** (replacement of the amidinium group by a dimethylamino group has been shown to afford similar selectivity with respect to DNA sequences[6]).

Scheme 2



Reagents and Conditions: **i) HNO_3 / Ac_2O / -40°C→ rt . ii) $MePPh_3Br$ / tBuOK / THF/ rt. iii) NBS / THF / -10°C→ rt. iv) MeONa / MeOH / rt. v) $Pd(OAc)_2$ / Et_3N / P(*o*-tol)$_3$ / DMF / 120 °C. vi) NaOH aq. / EtOH / 30°C. vii) WSCI / HOBt / DMF / rt. viii) HCl-Et_2O / MeOH. ix) $KMnO_4$ / Ac_2O / -5°C→ 10°C.**

The olefinic compound **8** was obtained from commercially available aldehyde **6** *via* 4-nitro derivative **7** prepared by nitration with nitric acid-acetic anhydride mixture[7], and following Wittig reaction. After several trials, it was found that Heck reaction[8] between olefin **8** and bromopyrrole **9** afforded the coupling product **10** in 62 % yield. The coupling reaction of carboxylic acid **11** derived from **10** (by alkaline hydrolysis) with a freshly prepared amine **12** (by catalytic hydrogenation of the corresponding nitro compound) afforded the target molecule **4** as a hydrochloride salt[9]. The special interest of the olefin intermediate **10** resides in its useful application to introduce α-diketo- function in the molecule by $KMnO_4$ oxidation[10]. Alkaline hydrolysis of **13** followed by the same procedure for **11** gave α-diketone product **5** as a hydrochloride salt[11].

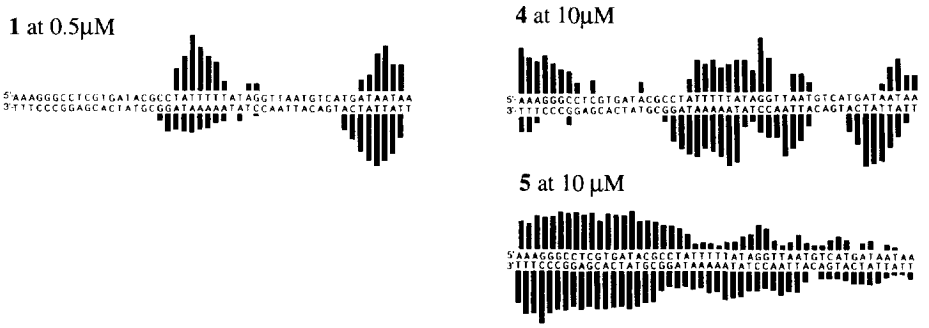
Binding properties of compounds (**1**-**5**) to DNA were evaluated with the ethidium displacement assay[12]. The calculated apparent binding constant to poly (dA-dT) and poly (dG-dC) are listed in Table 1. These data show that all compounds can bind to DNAs used. It is noteworthy that the nitro analogue **3** has weaker binding ability [8.7×10^5 M^{-1}] to poly (dA-dT) than **1** and **2** [345×10^5, 150.8×10^5 M^{-1}], but, binds stronger to poly (dG-dC) [3.5×10^5 M^{-1}] than **1** and **2** [1.3×10^5, 0.9×10^5 M^{-1}], indicating that the N-terminal nitro group affected for the affinity toward poly (dG-dC). The α-diketone linked analogue **5** has markedly improved acceptance of GC base pairs. The *K*app value of compound **5** for poly (dG-dC) [$20.9 \times 10^5 M^{-1}$] is close to that for poly (dA-dT) [$26.6 \times 10^5 M^{-1}$], while the *K*app value of compound **1** and **2** for poly (dG-dC) are about 2 orders of magnitude lower than those for poly (dA-dT).

Table 1. The apparent binding constants *K*app ($\times 10^5 M^{-1}$) determined by an ethidium displacement assay

	1	**2**	**3**	**4**	**5**
poly(dA-dT)	345(348)[4d]	150.8	8.7	16.9	26.6
poly(dG-dC)	1.3(2.0)[4d]	0.9	3.5	7.4	20.9

To further confirm the DNA binding interactions, MPE-Fe(II) footprinting experiments were carried out for **1**, **4** and **5** on the *Eco* RI / *Ssp* I restriction fragment of pBR 322 DNA[13]. The protection patterns from cleavage on bp 4301-4350 are shown in Figure 1.

Figure. 1. MPE protection patterns[a] for **1**,**4** and **5**.



a) Bar heights are proportional to the protection from cleavage at each band.

These results, in agreement with the data from the ethidium displacement assay, suggest the high selectivity of **1** for AT-rich sequences, the low acceptance of **4** for GC base pairs and the great tolerance of **5** for GC regions in their binding sites.

In conclusion, we demonstrated the synthesis of novel analogues of distamycin *via* intermolecular Heck reaction and $KMnO_4$ olefin oxidation as key reactions. It is also demonstrated that amide bond modifications of distamycin could lead to compounds with quite different DNA recognition patterns.

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9. Spectral data for **4** ^{1}H-NMR (270MHz, DMSO d-6) δ 1.78 (m, 2H), 2.56 (s, 6H), 2.79 (m, 2H), 3.24 (m, 2H), 3.73 (s, 3H), 3.81 (s, 3H), 3.89 (s, 3H), 6.65 (d, 1H, J = 16.2Hz), 6.90 (d, 1H, J = 1.7Hz), 6.94 (d, 1H, J = 1.7Hz), 6.98 (d, 1H, J = 16.2Hz), 7.15 (d, 1H, J = 1.7Hz), 7.18 (d, 1H, J = 1.7Hz), 7.20 (d, 1H, J = 1.7Hz), 7.97 (d, 1H, J = 1.7Hz), 8.16 (t, 1H, J = 5.3Hz), 9.98 (s, 1H), 10.36 (bs, 1H), FABMS (m/z) 482 (M-HCl+H), HRFABMS (m/z) 482.2522 (M-HCl+H, 482.2519 calcd for $C_{24}H_{32}N_7O_4$).
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11. Spectral data for **5** ^{1}H-NMR (270 MHz, DMSO-d 6) δ 1.87 (m, 2H), 2.74 (s, 3H), 2.76 (s, 3H), 3.03 (m, 2H), 3.24 (m, 2H), 3.82 (s, 3H), 3.94 (s, 3H), 4.07 (s, 3H), 6.92 (d, 1H, J = 2.0Hz), 7.22 (d, 1H, J = 1.7Hz), 7.38 (d, 1H, J = 2.0 Hz), 7.51 (d, 1H, J = 2.0Hz), 7.89 (d, 1H, J = 1.7Hz), 8.23 (bs, 1H), 8.52 (d, 1H, J = 1.7Hz), 10.12 (bs, 1H), 10.27 (s, 1H), FABMS (m/z) 512 (M-HCl+H), HRFABMS (m/z) 512.2238 (M-HCl+H), 512.2260 calcd for $C_{24}H_{30}N_7O_6$)
12. Morgan, A. R.; Lee, J. S.; Pulleyblank, D. F.; Murray, N. L.; Evans, D. H. *Nucl. Acids. Res.* **1979**, *7*, 547. These experiments followed conditions used in ref. 4 (d). Buffer solution (pH = 7.0) containing Tris-HCl (10 mM) and sodium chloride (10mM) was used.
13. A 50 μM MPE-Fe(II) solution was prepared by mixing a 100 μM MPE aqueous solution (20 μL) with a freshly prepared 100 μM ferrous ammonium sulfate aqueous solution (20 μL). Reaction mixture (16 μL) containing each compound, the labeled restriction fragment, sonicated calf thymus DNA (0.4 μg), sodium chloride, and Tris-HCl (pH = 7.5) was incubated at 20 °C for 30 min. To each tube was added 2 μL of a 50 μM MPE- Fe(II) solution followed by 2 μL of 100 mM dithiothreitol. Final concentrations were 5μM [MPE-Fe(II)], 10mM (dithiothreitol), 50 mM (sodium chloride), and 20 mM (Tris-HCl). The solutions were incubated at 20 °C for 10 min, lyophilized and electrophopresed.

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