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Short communication

DNA-binding properties of pyrrolo[2,1-*c*][1,4]-benzodiazepine N10-C11 amidines

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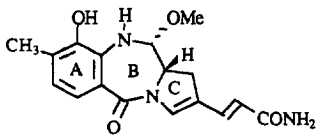
Summary – A series of pyrrolo[2,1-*c*][1,4]benzodiazepine N10-C11 amidines has been evaluated for in vitro DNA binding through thermal denaturation studies. Some of these compounds cause a significant increase in melting for calf thymus DNA (eg, 0.7 ± 0.1 °C for **21**), possibly due to non-covalent interaction with bases positioned on the floor of the minor groove in the DNA duplex.

pyrrolo[2,1-*c*][1,4]benzodiazepine / amidine / DNA-binding

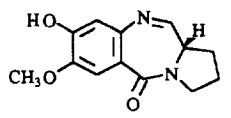
Introduction

The pyrrolo[2,1-*c*][1,4] benzodiazepines (PBDs) belong to a group of antitumor antibiotics which are biosynthetically derived from various *Streptomyces species*. Well-known members include anthramycin, tomaymycin, sibiromycin and DC-81 [1–3]. The antitumor activity of the PBDs is exerted through sequence-selective covalent binding to a C2-NH_2 group of guanine within the minor groove of double-stranded DNA via the electrophilic N10-C11 carbinolamine–imine functionality. It has been shown that the adducts span three base-pairs with a preference for Pu-G-Pu sequences [1, 2].

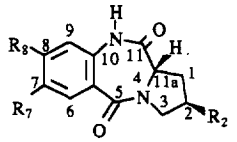
Although the N10-C11 carbinolamine moiety (or its equivalent) is responsible for the covalent (bonding) component of DNA interaction, other features of the molecule, including the overall 3-dimensional shape and the substituents in the A- and C-rings, may also contribute to the non-covalent interaction with duplex DNA. Furthermore, this non-covalent component may constitute part of the DNA-recognition process, leading to the preferred binding site of 5'-Pu-G-Pu [4]. The sequence-preferential binding properties of a C8-C8-linked PBD dimer have recently been examined using high-resolution NMR and molecular modelling techniques [5], confirming a 5'-Pu/Py-GA bonding preference for each PBD subunit.



Anthramycin
1



DC-81
2



	R_7	R_8	R_2
3a	CH_3O	OH	OH
3b	CH_3O	CH_3COO	$OCOCH_3$
3c	H	H	H
3d	H	H	OH

A recent study showed for the first time that PBD dilactams of type **3a** and **3b** substituted with either hydroxyl or acetoxyl groups at the C2- or C8-positions could bind strongly to DNA as measured by thermal denaturation studies. They increased the melting temperature of calf thymus DNA by 3.3 and 2.9 °C respectively [4]. From a study of a series of compounds, useful structure–activity (SAR) information was obtained, including the fact that the unsubstituted parent dilactam **3c** or the monosubstituted

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hydroxyl analogue **3d** failed to bind to DNA to a significant extent. It was also established that the *R*-configuration at C2 is essential for DNA interaction, and that bulky derivatives of the C2- and C8-hydroxyls can inhibit interaction. We now report that conversion of the N10-C11 amide moiety of the PBD system to an amidine functionality can lead to significant DNA interaction, even in cases where the N10-C11 amide equivalent does not bind (eg, **3d** with one hydroxyl at C2).

Chemistry

Although several groups have demonstrated the lack of reactivity of a lactam moiety when at the N10-C11 position of a PBD [4, 6, 7], it can easily be converted to a thiolactam which appears to be more chemically reactive [8, 9]. We have recently demonstrated that, in mild conditions, thiolactams of type **5** and **6** react with primary amines in the presence of mercuric chloride to give amidines of type **8–12** [10, 11]. This method has been extended to benzodiazepines with unsubstituted pyrrolidine rings. Starting from dithiolactam **4**, we obtained the amidine **7** using ammonia. The dithiolactam **4** has been synthesized from the corresponding dilactam **3c** [12] using Lawesson's reagent. No isomerization occurred during this reaction. The unsubstituted simple amidines **8**, **9** are stable but react with isocyanate in boiling toluene to give, for example, ureas of type **16** and **17** [11]. In the same manner, the amidine **7** treated with different isocyanates in boiling toluene converted to ureas **13–15**. With acid chlorides in dry tetrahydrofuran (THF) in the presence of triethylamine (TEA) at room temperature, the unsubstituted amides **8**, **9** gave amides of type **18**, **19** [11]. Treatment of iminothioether **20** with hydrazine afforded the hydrazidine **21** [11] (scheme 1).

The DNA-binding affinity of these amidines has been assessed by thermal denaturation studies using calf thymus DNA. Aqueous solutions of DNA were prepared in Millipore-purified water buffered at pH 7.00 ± 0.01, containing 10 μmol·dm^{-3} sodium phosphate and 1 μmol·dm-3 ethylenediaminetetraacetic acid (EDTA). No added salt or support electrolyte was used. Working solutions containing 100 μmol·dm^{-3} DNA alone and in the presence of 20 μmol·dm^{-3} of the compounds were monitored using a Shimadzu UV-2101 PC spectrophotometer fitted with a Peltier heating accessory, following incubation at 37.0 ± 0.1 °C for 18 h. The sample was heated at 1 °C·min^{-1} until thermal denaturation was complete, as judged by an increase in optical absorption at 260 nm. Thermal denaturation temperatures (*Tm*) were determined at a relative absorbance value of 0.50 and are reported as the mean ± SEM of three or four determinations. The change in temperature (ΔTm) following interaction of DNA with an added compound is given by: $\Delta Tm = Tm_{DNA\text{-}drug} - Tm_{DNA}$ and is reported in table I for a fixed [DNA]:[drug] ratio of 5:1.

Results and discussion

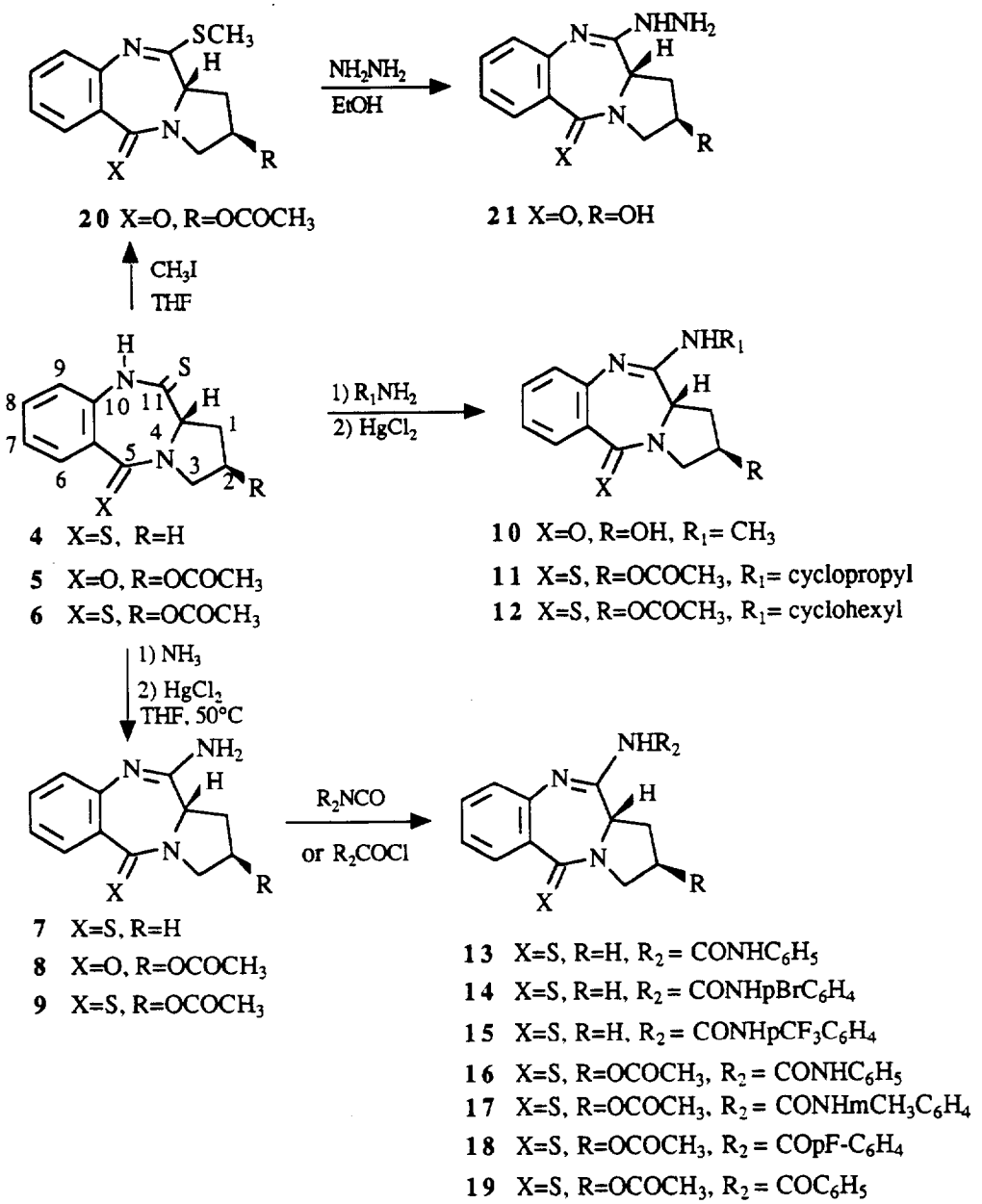
The results presented in table I show that nearly all of the 11 amidine derivatives examined significantly elevate the temperature of the DNA helix-to-coil melting transition. That is, the compounds effect differential stabilization of the DNA duplex form. All compounds possess an affinity for DNA similar to that of the natural product DC-81, which binds covalently to DNA. Interestingly, both **10** and **21** have C2-hydroxyl groups in the *R*-configuration, and so can be directly compared to the PBD dilactam **3d** which does not bind significantly to DNA. The DNA-binding affinity of **10** and **21** must therefore be mostly due to the N10-C11 amidine functionality. Compound **13** is the first reported example of a PBD with a C5-thioketone substituent binding to DNA. Even though it has no C2-substituent this analogue still raises the DNA melting point significantly. For dilactams **3a** and **3b**, it was possible to develop a model to rationalize their DNA-binding in terms of a complex with the molecules positioned in the minor groove of duplex DNA and with the N10-C11 amide moiety directed towards the floor of the groove [4]. Of the amidines studied that bind to DNA with a $\Delta Tm > 0.6$ °C, it is possible that a similar mode of binding occurs, as there may be sufficient space to accommodate the amidine moiety within the minor groove. However, as with the previous study [4], those compounds with $\Delta Tm < 0.6$ °C may be interacting with DNA through non-specific electrostatic interactions with the phosphate backbone of DNA, particularly as the amidines are likely to be charged at the pH of the buffer used for the experiment. It is also possible that ionization of the amidine groups on some of the molecules examined may influence their DNA-binding affinity.

These results suggest that an N10-C11 amidine moiety can play an important role in the non-covalent DNA binding of PBD dilactams. Other compounds in this series are under investigation in a continuation of our structure–activity studies.

Experimental protocols

Chemistry

Melting points were determined on a Köfler bank apparatus and are uncorrected. Infrared spectra (KBr disks) were recorded on a Philips PU 9716 spectrophotometer (υ_{max} in cm^{-1}). ^{1}H-NMR spectra were recorded on a JEOL JNM-FX 200 with tetramethylsilane as the internal standard, using DMSO-d_6 as



Scheme 1.

solvent. Chemical shifts data are reported in parts per million (δ in ppm) and bs, s, d, m designate broad singlet, singlet, doublet and multiplet respectively. Experimental protocols for the synthesis of compounds **8**, **9** and **10–12**, **16–21** are described in references [10] and [11].

(11aS)-1,2,3,10,11,11a-Hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5,11-dithione **4**

To a suspension of dilactam **3c** (0.046 mol) in 1,4-dioxane (250 mL) was added Lawesson's reagent (22.47 g, 0.05 mol). The reaction mixture was heated under reflux for 5 h. After cooling, the solid formed was separated, dried and recrystallized from 1,4-dioxane to give **4** as a yellow solid. Yield: 57%, mp > 260 °C, IR: 3100, 3060 (NH). ^{1}H-NMR δ: 2.00–2.28 (m, 3H, $1H_1$ and H_2), 2.80–2.92 (m, 1H, $1H_1$), 3.79–3.93 (m, 2H, H_3), 4.57 (d, 1H, H_{11a}, J = 6.35 Hz), 7.24 (t, 1H, J = 7.81 Hz), 7.29 (t, 1H, J = 7.81 Hz), 7.53 (t, 1H, H7, J = 6.83 Hz), 8.12 (d, 1H, H6, J = 6.84 Hz), 12.61 (s, 1H, NH). Anal calc for $C_{12}H_{12}N_2S_2$: C, 58.03, H, 4.87, N, 11.28; found C, 58.18, H, 5.07, N, 11.13.

Table I. Thermal denaturation of calf thymus DNA by amidine compounds.

Compound	*ΔTm (°C) after incubation for 18 h*
DC-81	0.65 ± 0.14[b]
3c	< 0.5
3d	< 0.5
10	0.7 ± 0.1[b]
11	0.6 ± 0.1[a]
12	0.6 ± 0.1[a]
13	0.7 ± 0.1[b]
14	0.6 ± 0.1[a]
15	0.6 ± 0.1[a]
16	0.6 ± 0.1[b]
17	0.5 ± 0.1[b]
18	0.5 ± 0.1[b]
19	0.5 ± 0.1[b]
21	0.7 ± 0.1[a]

[a,b]Mean of 3, 4 measurements respectively.

(11aS)-1,2,3,4-Tetrahydro-11-amino-5H-pyrrolo[2,1-c][1,4]-benzodiazepine-5-thione **7**

Dithiolactam **4** (4.03 mmol) was dissolved in dry tetrahydrofuran (50 mL) and warmed to 55 °C. Into this solution was bubbled an ammonia stream for 10 min followed by the addition of mercury chloride (6.04 mmol, 1.64 g, 1.5 eq). The reaction mixture became black and was stirred for 1 h and then filtered; the filtrate was evaporated in vacuo and the residue partitioned between ethyl acetate and saturated sodium thiosulfate solution. The organic layer was separated and washed successively with saturated sodium thiosulfate and brine, dried (magnesium sulfate) and concentrated in vacuo to give a yellow solid which was recrystallized from ether to give **7**. Yield: 61%, mp: 150 °C, IR: 3380, 3350 (NH), 1645 (C=N). ^{1}H-NMR δ: 2.07–2.25 (m, 3H, $1H_1$ and H_2), 2.50 (m, 1H, $1H_1$), 3.62 (m, 1H, $1H_3$), 3.98 (d, 1H, $1H_3$, J = 6.35 Hz), 4.06 (d, 1H, H_{11a}, J = 6.83 Hz), 6.92 (t, 1H, J = 7.81 Hz), 6.94 (t, 1H, J = 7.82 Hz), 7.13 (bs, 2H, NH_2), 7.32 (t, 1H, H_7, J = 7.82 Hz), 8.05 (d, 1H, H_6, J = 7.81 Hz). Anal calc for $C_{12}H_{13}N_3S$: C, 62.31, H, 5.66, N, 18.16; found C, 62.45, H, 5.48, N, 18.29.

General procedure for the synthesis of ureas ***13, 14 and 15***

To a suspension of the amidine **7** (4.32 mmol) in toluene (30 mL) was added the corresponding isocyanate (4.32 mmol, 1 eq). The reaction mixture was heated under reflux until a solution was obtained. The reflux was then continued for 30 min. The toluene was evaporated in vacuo and the solid residue washed with ether and recrystallized from toluene.

(11aS)-1,2,3,4-Tetrahydro-11-(N,N'-phenylureido)-5H-pyrrolo-[2,1-c][1,4]benzodiazepine-5-thione ***13.*** Yield: 67%, mp: 224 °C, IR: 3250 (NH), 1620 (C=O and C=N). ^{1}H-NMR δ: 2.07–2.22 (m, 3H, $1H_1$ and H_2), 2.82 (m, 1H, $1H_1$), 3.75 and 4.02 (m, 2H, H_3), 4.61 (d, 1H, H_{11a}, J = 5.37 Hz), 6.97–7.33 (m, 5H), 7.50 (t, 1H, J = 7.81 Hz), 7.67 (d, 2H, J = 7.81 Hz), 8.11 (d, 1H, H_6, J = 8.30 Hz), 9.74 (s, 1H, NH), 11.64 (s, 1H, NH). Anal calc for $C_{19}H_{18}N_4OS$: C, 65.12, H, 5.18, N, 15.99; found C, 64.92, H, 5.35, N, 15.75.

(11aS)-1,2,3,4-Tetrahydro-11-(N,N'-4-bromo-phenylureido)-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-thione ***14.*** Yield: 70%, mp: 212 °C, IR: 3280 (NH), 1625 (C=O and C=N). ^{1}H-NMR δ: 2.05–2.27 (m, 3H, 1 H_1 and H_2), 2.80 (m, 1H, $1H_1$), 3.75 and 4.01 (m, 2H, H_3), 4.61 (d, 1H, H_{11a}, J = 5.85 Hz), 7.19–7.68 (m, 7H), 8.11 (d, 1H, H_6, J = 7.81 Hz), 9.88 (s, 1H, NH), 11.64 (s, 1H, NH). Anal calc for $C_{19}H_{17}N_4OSBr$: C, 53.15, H, 3.99, N, 13.05; found C, 52.90, H, 4.11, N, 12.91.

(11aS)-1,2,3,4-Tetrahydro-11-(N,N'-4-trifluoromethylphenyl-ureido)-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-thione ***15.*** Yield: 63%, mp: 224 °C, IR: 3260 (NH), 1630 (C=O), 1600 (C=N). ^{1}H-NMR δ: 2.08–2.29 (m, 3H, $1H_1$ and H_2), 2.80 (m, 1H, $1H_1$), 3.80 and 4.03 (m, 2H, H_3), 4.62 (d, 1H, H_{11a}, J = 5.85 Hz), 7.21–7.91 (m, 7H), 8.12 (d, 1H, H_6, J = 7.82 Hz), 10.10 (s, 1H, NH), 11.62 (s,1H, NH). Anal calc for $C_{20}H_{17}N_4OSF_3$: C, 57.41, H, 4.09, N, 13.39; found C, 57.68, H, 4.28, N, 13.23.

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