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Bioorganic & Medicinal Chemistry Letters 14 (2004) 4107–4111
Bioorganic & Medicinal Chemistry Letters

Synthesis of C8-linked pyrrolo[2,1-*c*][1,4]benzodiazepine-acridone/acridine hybrids as potential DNA-binding agents

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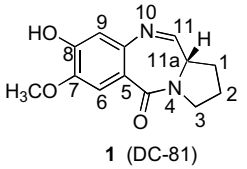
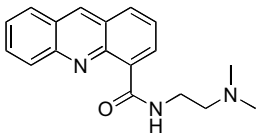
Received 1 March 2004; accepted 13 April 2004

Abstract—Pyrrolobenzodiazepine hybrids linked to acridone/acridine ring systems at C8-position have been designed and prepared that exhibit significant DNA-binding affinity, and a representative compound shows promising in vitro anticancer activity.

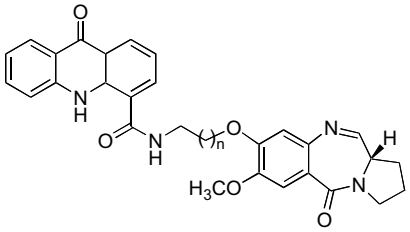
There is a considerable interest in the development of low molecular weight DNA-binding agents towards their application on various biological responses particularly anticancer activity. Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a group of potent DNA-interactive antitumour antibiotics derived from *Streptomyces* species,[1] well known members include DC-81 (**1**), anthramycin, chicamycin and tomaymycin. Their interaction with DNA has been extensively investigated and it is considered unique since they bind within the minor groove of duplex DNA forming a covalent aminal bond with N2-amino group of guanine base,[2] giving rise to preference for Pu–G–Pu sequences.[3] The PBDs have also been used as a scaffold to attach ethylenediaminetetraacetic acid (EDTA),[4] epoxide,[5] polyamide,[6] oligopyrrol[7] and cyclic amine[8] moieties leading to novel hybrids of PBD. Recently, we have also designed and synthesized a number of PBD hybrids that have significant DNA-binding ability and potent anticancer activity.[9]



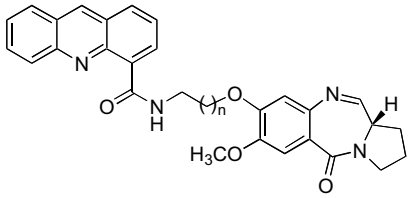
1 (DC-81)



2 (DACA)



3 a-b n = 1, 2



4 a-b n = 1, 2

Keywords: Pyrrolobenzodiazepine; Acridone; Acridine; DNA-binding affinity; In vitro anticancer activity.
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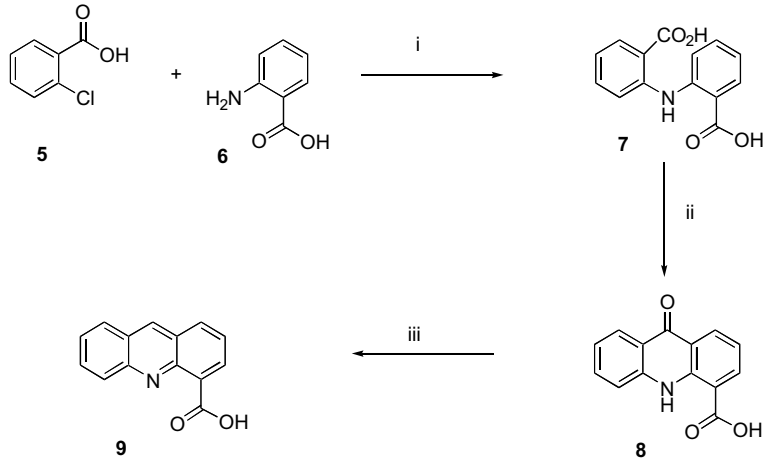
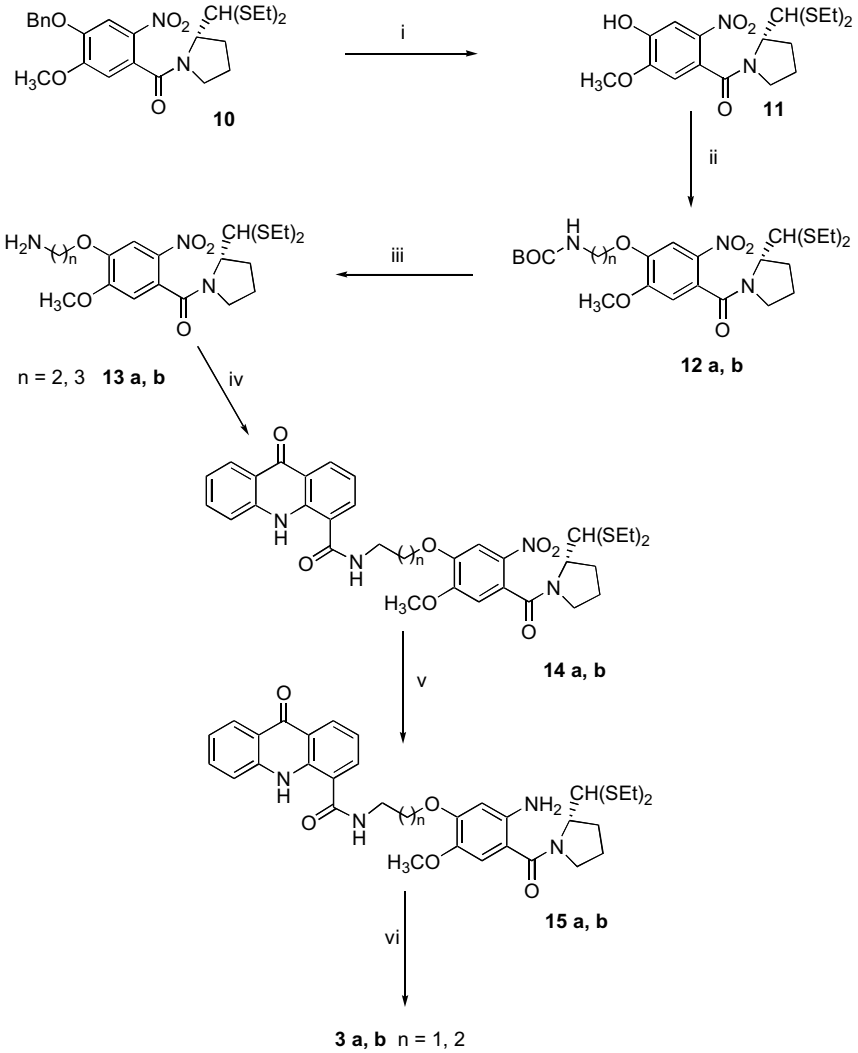
doi:10.1016/j.bmcl.2004.04.087



Scheme 1. Reagents and conditions: (i) K_2CO_3, Cu, pentylalcohol, reflux, 36 h, 85%; (ii) H_2SO_4, 100 °C, 4 h, 80%; (iii) Al–Hg, KOH, aqueous ethanol, reflux, then $FeCl_3$, 60%.



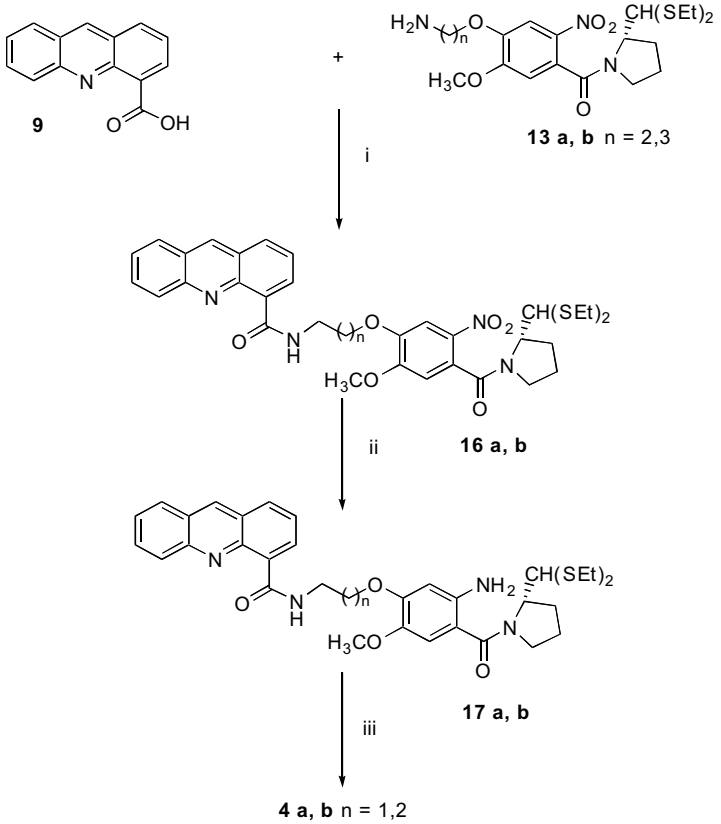
Scheme 2. Reagents and conditions: (i) EtSH–BF_3OEt_2, CH_2Cl_2, 12 h, rt, 70%; (ii) Boc-protected bromo alkylamine, K_2CO_3, DMF, rt, 24 h, 85–88%; (iii) TFA, CH_2Cl_2, 8 h, rt; (iv) EDCl–HOBt, compound **8**, DMF, 24 h, rt, 50–54%; (v) $SnCl_2$–$2H_2O$, MeOH, reflux, 2 h, 70–73%; (vi) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 48–52%.

Acridine and acridone based compounds comprise of an important class of DNA-intercalating anticancer drugs, and are structurally characterized by the presence of a planar and semiplanar chromophore portion possibly

capable of intercalation into DNA. These compounds are known to possess a broad range of biological activities with different modes of action, but all have in common the ability to bind tightly but reversibly to DNA by intercalation between the base pairs of the double helix. In fact, the concept of intercalation has been first introduced to explain the reversible and non-covalent binding interactions between acridines and DNA.[10] Some noticeable examples that are clinically in use and in clinical trials are constituted by amsacrine,[11] its derivative CI-921[12] and *N*-[(2-dimethyl amino)-ethyl]acridine-4-carboxamide (DACA).[13] DACA (**2**) is a lipophilic mono-intercalator, which entered phase I clinical trial on the basis of its mixed inhibition of both DNA topoisomerase-I and topoisomerse-II, and excellent activity in experimental solid tumour models.[14] Further, DACA has shown to retain activity in cell lines resistant due to alterations in topo-I, topo-II and *p*-glycoprotein expressions.[15] A number of acridine derivatives have exhibited interesting antitumour properties, including the acridone-4-carboxamide,[16] and the bis-functionalized acridone/acridine-4-carboxamides.[17]

Therefore, it has been considered of interest to design and synthesize C8-linked PBD-acridone/acridine hybrids that could not only improve the DNA-binding ability but also the biological activity.[18] This is in continuation of our efforts in structural modifications of PBD ring system and also the development of new synthetic strategies[19] for this ring system. We herein report the synthesis and DNA-binding ability of C8-linked acridone/acridine PBD hybrids with different alkoxyamide spacers.

Synthesis of these acridone/acridine linked PBD hybrids has been carried out by employing the corresponding acridone/acridine 4-carboxylic acids as starting materials. Acridine-4-carboxylic acid **9** has been prepared by reduction of the corresponding acridone-4-carboxylic acid **8** with aluminium mercury amalgam, followed by $FeCl_3$ reoxidation of the resulting acridane.[13] Jourdan–Ullmann copper-catalyzed condensation of 2-chloro benzoic acid **5** with anthranilic acid[12] **6** followed by cyclization of the resulting diphenyl amine-2,2′-dicarboxylic acid[20] **7** in H_2SO_4 gives the required acridone 4-carboxylic acid **8** (Scheme 1). Whereas, (2*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal **10** has been prepared by literature method,[21] which upon debenzylation gives **11**. Linker components *N*-(*tert*-butoxycarbonyl)bromoalkyl amines have been prepared by treatment of bromoalkyl amines with Boc-anhydride. These have been linked to **11** through an ether linkage using K_2CO_3, in DMF at room temperature to give **12a**–**b**. Their deprotection with TFA affords the required precursor amines (**13a**–**b**), followed by amidation with acridone or acridine-4-carboxylic acids using EDCI/HOBT affords the corresponding nitrothioacetal intermediates **14a**–**b**/**16a**–**b**. Further, these upon



Scheme 3. Reagents and conditions: (i) EDCl–HOBt, DMF, 24 h, rt, 56–58%; (ii) $SnCl_2$–$2H_2O$, MeOH, reflux, 2 h, 68–72%; (iii) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 49–53%.

reduction by $SnCl_2 \cdot 2H_2O$ in methanol and followed by deprotection of aminothioacetal precursors **15a–b/17a–b** using $HgCl_2/CaCO_3$ affords the acridone/acridine linked PBD hybrids **3a–b/4a–b** (Schemes 2 and 3).[22]

The DNA-binding ability of these C8-linked PBD-acridone/acridine hybrids has been investigated by thermal denaturation studies using calf thymus (CT) DNA[23] at pH 7.0, incubated at 37 °C. It is interesting to observe that these hybrid molecules elevate the helix melting temperature of the CT-DNA significantly (Table 1). One of these hybrids (**3b**) elevates the helix melting temperature of CT-DNA by 12.5 °C after incubation for 18 h. In the same experiment the naturally occurring DC-81 having one imine group exhibits ΔT_m of 0.7 °C. The enhancement of DNA-binding ability of these hybrids can be correlated to other interactions produced by the acridone/acridine component in addition to covalent linkage of the imine component. Moreover, as the carbon chain increases from two to three in case of **3b** there is a substantial increase in the DNA-binding affinity.

One of the representative compound **3b** has been evaluated for in vitro anticancer activity in the standard 60-cancer cell line screen of NCI (Table 2). The LC_{50} value of compound **3b** against nonsmall cell lung cancer NCI-H23 cell line is 0.07 μM. It exhibits activity in melanoma cancer panel, in which M14 and UACC-62 cell lines are affected, with LC_{50} values of <0.01 μM. It also exhibits cytotoxic potency against renal cancer cell line A498 with LC_{50} value of 0.05 μM. Further, this analogue exhibits less than 10 nM potency at the GI_{50} level against most of the cancer cell lines. The in vitro cytotoxicity (IC_{50}) for the naturally occurring DC-81[24] is 0.38 and 0.33 μM in L1210 and PC6 cell lines, respectively. Interestingly this compound has promising in vitro anticancer activity in a number of human cancer cell lines.

Table 1. Thermal denaturation data for C8-linked acridone/acridine PBD hybrids with calf thymus (CT) DNA

PBD hybrids	[PBD]:[DNA] molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C for	
		0 h	18 h
3a	1:5	4.7	5.7
3b	1:5	11.0	12.5
4a	1:5	3.1	5.0
4b	1:5	5.9	7.7
DC-81	1:5	0.4	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, $T_m = 69.0$ °C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ±0.1–0.2 °C.

[b] For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

Table 2. In vitro cytotoxicity of **3b** in selected cancer cell lines

Cancer panel/cell line	LC_{50} (μM)
Nonsmall cell lung	
NCI-H23	0.07
Melanoma	
M14	<0.01
UACC-62	<0.01
Renal	
A498	0.05

In conclusion, C8-linked PBD-acridone/acridine hybrids have been synthesized that exhibit enhanced DNA-binding ability particularly for **3b** and promising anticancer activity for this representative example in certain cancer cell lines. This investigation further reveals the significance of combining a noncovalent DNA-binding component (acridone) to the covalent binding PBD moiety. The detailed mechanistic and molecular modelling studies for these PBD hybrids are in progress.

Acknowledgements

We thank the National Cancer Institute (NCI), Maryland for the primary anticancer assay in human cancer cell lines. We are also grateful to CSIR, New Delhi for the award of research fellowships to O.S., P.R., G.R. and P.P.K.

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22. Selected data for compound **3a**. ^{1}H NMR ($CDCl_3$) δ 1.90–2.35 (m, 4H), 3.35–3.70 (m, 4H), 3.80 (s, 3H), 4.10–4.30 (m, 3H), 6.80 (s, 1H), 7.20–7.35 (m, 3H), 7.50–7.70 (m, 3H), 8.20 (d, 2H, $J = 8.18$ Hz), 8.40 (d, 1H, $J = 8.2$ Hz), 9.05–9.10 (m, 1H), 12.60 (s, 1H); MS (FAB) 511 $[M+H]^{+\cdot}$.
Compound **3b**. ^{1}H NMR ($CDCl_3$) δ 2.0–2.40 (m, 6H), 3.50–3.75 (m, 4H), 3.80 (s, 3H), 4.20–4.40 (m, 3H), 6.80 (s, 1H), 7.20–7.30 (m, 2H), 7.40–7.45 (m, 1H), 7.50 (s, 1H), 7.60–7.80 (m, 3H), 8.10 (d, 1H, $J = 8.18$ Hz), 8.40 (d, 1H, $J = 8.2$ Hz), 8.70 (d, 1H, $J = 7.6$ Hz), 12.30 (s, 1H); MS (FAB) 525 $[M+H]^{+\cdot}$.
Compound **4a**. ^{1}H NMR ($CDCl_3$) δ 1.90–2.35 (m, 4H), 3.40–3.80 (m, 4H), 3.90 (s, 3H), 4.10–4.40 (m, 3H), 6.80 (s, 1H), 7.45–7.80 (m, 5H), 7.95 (d, 1H, $J = 8.18$ Hz), 8.10 (d, 1H, $J = 8.18$ Hz), 8.20 (d, 1H, $J = 8.92$ Hz), 8.80 (s, 1H), 8.90 (d, 1H, $J = 6.69$ Hz), 12.30 (s, 1H); MS (FAB) 495 $[M+H]^{+\cdot}$. Compound **4b**. ^{1}H NMR ($CDCl_3$) δ 2.0–2.45 (m, 6H), 3.50–3.70 (m, 2H), 3.80–4.0 (m, 5H), 4.25–4.45 (m, 3H), 6.80 (s, 1H), 7.50–7.80 (m, 5H), 8.0–8.10 (m, 2H), 8.20 (d, 1H, $J = 8.18$ Hz), 8.9 (s, 1H), 9.0 (d, 1H, $J = 7.43$ Hz), 12.0 (s, 1H); MS (FAB) 509 $[M+H]^{+\cdot}$.
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