Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 669–674

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

INTRODUCTION OF A NITROXIDE GROUP ON POSITION 2 OF 9-PHENOXYACRIDINE: EASY ACCESS TO SPIN LABELLED DNA-BINDING CONJUGATES

Philippe Belmont, Christian Chapelle, Martine Demeunynck*, Josette Michon*, Pierre Michon and Jean Lhomme

LEDSS, CNRS/Université Joseph Fourier, BP 53, 38041 Grenoble Cédex 9, France

Received 16 October 1997; accepted 9 February 1998

Abstract. In the search for spin labelled intercalators of general use to construct DNA-binding conjugates, 6-chloro-2-[(1-oxyl-2,2,5,5-tetramethyl-pyrrolin-3-yl)methyloxy]-9-phenoxy-acridine **5**, has been prepared. This key-intermediate reacts with amines to give the corresponding labelled 9-amino substituted acridines. Comparative EPR and fluorescence measurements show that the label causes only little modification of the binding properties of acridine.

Introduction

Spin labelling constitutes a powerful approach to extract structural and dynamic information on the binding of molecules to their biological receptor. It has been successfully applied in the field of nucleic acids to study the interaction of labelled intercalators (acridines,[1] ethidium,[2] propidium,[3] aminofluorene,[4] psoralens[5]...) and groove binding drugs (oxazolopyridocarbazole,[6] ruthenium complexes[7]...) with oligo- and polynucleotides and to study their fate in the cell.[8] It has also been used to study the hybridization of labelled oligonucleotides to the complementary DNA sequence.[9] The approach requires introduction of the label, generally a nitroxide, at a site of the drug that is not involved in the binding. Acridines represent a typical class of strong DNA intercalators and the 2-methoxy-6-chloro-9-aminoacridine nucleus present in the antimalarial drug quinacrine[10] probably represents one of the most widely used structural unit to construct DNA-binding conjugates (see for example the acridine-oligonucleotide conjugates in the antisense strategies[11]). In the intercalation complex that it forms with DNA[12] the bulky methoxy group protrudes outside the groove so that replacement of the methyl by a methylene pyrroline-1-oxyl group should bring little modification to the binding. We report here preparation of the key labelled intermediate **5** that can be conveniently linked to the properly amino containing unit to give the required labelled 9-amino acridine conjugate. As an illustration conjugate **8** was prepared along with the model propylamino-acridine **6**, and shown to behave quite closely to the unlabelled DNA cleavage agent **10**.[12,13]

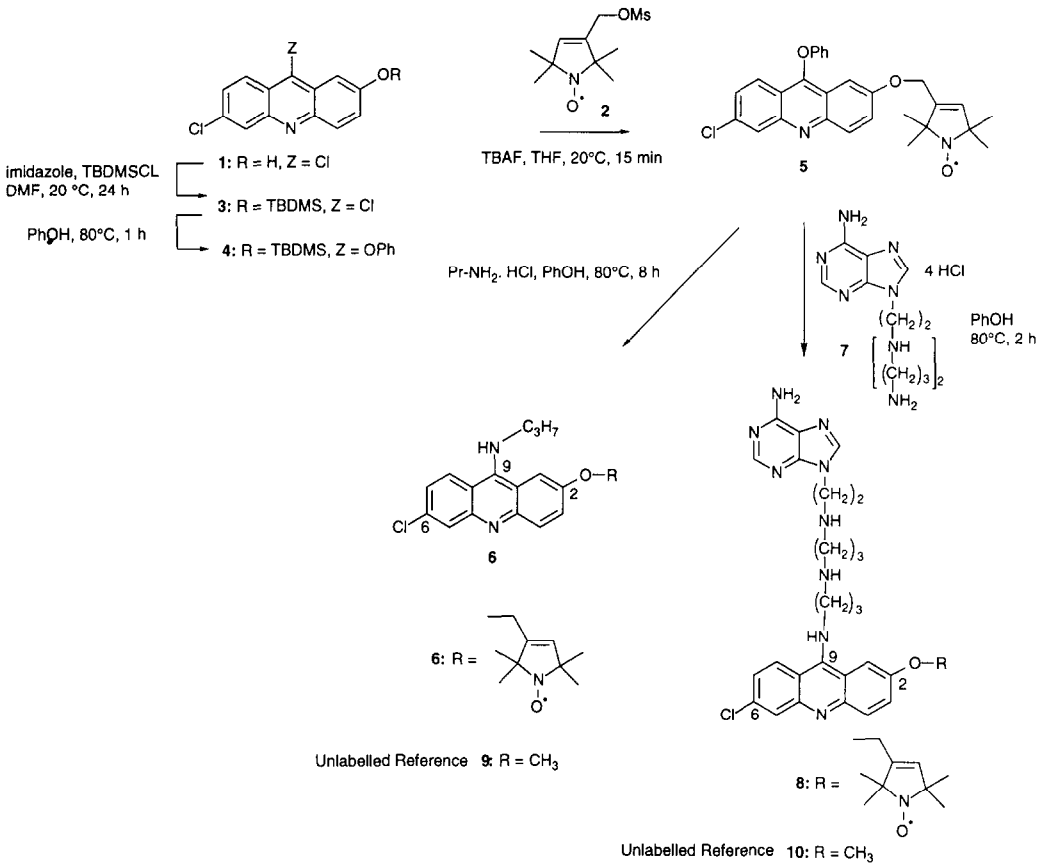
Results and discussion

Silyl protection of the 2-hydroxyl in **1**[14] allowed clean substitution of the 9-chloro substituent by a phenoxy group to afford **4**, upon which the nitroxide label could be grafted by treatment with the mesylated

* Authors to whom correspondance should be addressed. E-mail: Martine.Demeunynck@ujf-grenoble.fr

0960-894X/98/$19.00
PII: S0960-894X(98)00089-4

derivative **2**[15] in the presence of tetrabutylammonium fluoride. The phenoxy leaving group in the key intermediate **5** could be easily substituted by heating respectively with propylamine and amino derivative **7** to give the aminoacridines **6** and **8**.



Scheme 1

In diluted aqueous buffer solutions **6** and **8** exhibited the characteristic triplet (16 G splitting) of the nitroxide radical undergoing fairly rapid motion. Correlation time $\tau_c = 9x10^{-11}$ s and $\tau_c = 1.3x10^{-10}$ s were calculated for **6** and **8** respectively, using the Kivelson's equation.[16] These values characterize the drugs "free" in solution. In the presence of calf thymus DNA the spectra observed for the two compounds were the summation of two spectra, one corresponding to the radicals free in solution, the other to the radicals in interaction with DNA (Figure 1). The latter, as determined by subtraction, were broad and asymmetric showing a maximum hyperfine splitting of 48 G. Identical τ_c values ($\tau_c = 6x10^{-9}$ s), indicative of slow motion, were determined for **6** and **8** by comparison with the calculated spectra.[17]

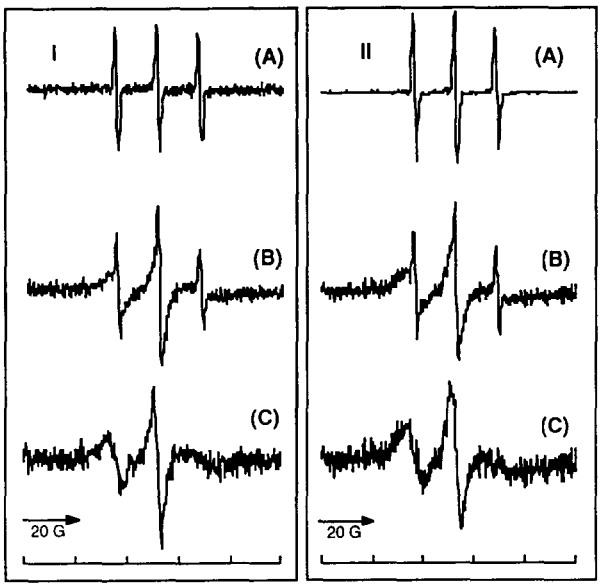


Figure 1. EPR spectra of compounds **6** (I) and **8** (II) in the absence (A) and presence (B) of DNA and difference spectra (C). Solutions were made in 25 mM Tris-HCl, pH 7, 0.1 M NaCl, 0.2mM EDTA solutions containing 2 % of DMSO. Due to the difference of binding affinity of the two compounds, different concentration ranges were used. For **6**, the DNA concentration was 1 10^{-3} M in base pairs and the drug concentration 1 10^{-5} M. For **8**, the DNA concentration was 1 10^{-4} M in base pairs and the drug concentration 1.5 10^{-5} M.

The EPR spectra registered at various drug/DNA concentrations and treated according to Mc Ghee and Von Hippel[18] allowed determination of the binding constants that appeared fairly similar to those determined independently by the fluorescence spectra (Table 1). The labelled molecule **8** was also used to determine by EPR the binding constant of the unlabelled compound **10** according to the competition technique routinely used for fluorescence spectroscopy. Again, the value measured fits the value obtained by fluorescence.

	Propyl Acridine		Adenine-Acridine Conjugate	
	9	**6**	**10**	**8**
τ_c (s)[a]		9×10^{-11}		1.3×10^{-10}
τ_c (s)[b]		6×10^{-9}		6×10^{-9}
K_{fluo}	6.4×10^{4}	3×10^{4}	4×10^{5}	1.5×10^{5}
K_{EPR}		2×10^{4}	4×10^{5} [c]	2×10^{5}

Table 1. a) τ_c of the free drug; b) τ_c in the presence of DNA; c) value measured by displacement of labelled compound **8.**

Conclusion

The most important result that emerges from the present study (Table 1) is comparison between labelled and unlabelled derivatives (**6** and **8** versus **9** and **10**) which indicates that the binding constant is only little modified by the label, being slightly lower for the labelled molecules. This shows the potential of the key label **5** to be introduced into DNA-binding conjugates in a large field of applications in structural and biological studies.

Experimental section

2-*tert*-butyldimethylsilyloxy-6,9-dichloro-acridine 3. To a mixture of hydroxyacridine **1** (0.7 g, 0.65 mmol) and imidazole (0.32 g, 4.66 mmol) dissolved in dimethylformamide (25 mL) was added *tert*-butyldimethylsilylchloride (0.53 g, 3.5 mmol). The solution was stirred at room temperature under nitrogen for 24 h. The solution was then filtered and water was added until precipitation. The desired compound **3** was filtered off and crystallised from a methanol-water mixture (0.47 g, 47 %), mp 91-92°C, elemental analysis calcd for $C_{19}H_{21}NOSiCl_2$: C, 60.31; H, 5.59; N, 3.70; Si, 7.42; found C, 61.16; H, 5.72; N, 3.65; Si, 7.47; IR (KBr) 3000-2700, 1600, 1440, 1410, 1390, 1290, 1210, 1110, 1055, 1000, 980, 945, 910, 860, 840, 830, 800, 770, 680, 650 and 620 cm^{-1}; ^1H NMR (200 MHz; DMSO-d_6) δ_H 0.07 (6H, s, $Si(CH_3)_2$), 1.00 (9H, s, $SiC(CH_3)_3$), 7.60-7.75 (3H, m, Acr-*H*), 8.15-8.35 (3H, m, Acr-*H*); MS (FAB(+))*m/z* 379 $(M+1)^+$; UV/Vis (EtOH): λ_{max} (ε) 266 (201000), 333 (4536), 356 (6804), 398 (5812), 417 (5812).

2-*tert*-butyldimethylsilyloxy-6-chloro-9-phenoxy-acridine 4. Chloro derivative **3** (0.4 g, 1.06 mmol) was stirred in phenol (10 mL) at 80 °C for 1 h. The reaction mixture was then cooled to room temperature and poured into diethyl ether. The solid was filtered and crystallised from acetone. [The free base could be obtained by treatment with 1 N sodium hydroxide and extraction with diethyl ether. The organic layer was washed with water and dried on sodium sulphate, the resulting solution was concentrated until compound **4** precipitated (0.37 g, 80 %)], mp 143-145 °C, elemental analysis calcd for $C_{25}H_{26}NO_2SiCl,1HCl,0.5\ H_2O$: C, 62.31; H, 5.81; N, 2.91; found: C, 62.05; H, 5.77; N, 3.02; ^1H NMR (200 MHz; $CDCl_3$) δ_H 0.06 (6H, s, $Si(CH_3)_2$), 0.90 (9H, s, $SiC(CH_3)_3$), 6.80-6.82 (2H, m), 7.00-7.05 (1H, m), 7.19-7.30 (3H, m), 7.35-7.45 (2H, m), 8.02 (1H, d, J = 9 Hz), 8.10 (1H, d, J = 9.5 Hz), 8.20 (1H, m); IR (KBr) 3015, 2920, 2840, 1645, 1590, 1460, 1410, 1350, 1270, 1230, 1200, 1060, 990, 930, 870, 840, 830, 800, 775, 750, 710, 690, 670 and 625 cm^{-1}; MS (FAB(+))*m/z* 437 $(M+1)^+$; UV/Vis (EtOH): λ_{max} (ε) 267 (441200), 338 (3589), 383 (4745), 405 (4307).

6-chloro-2-[(1-oxyl-2,2,5,5-tetramethyl-pyrrolin-3-yl)methyloxy]-9-phenoxy-acridine 5. Phenoxy silyl derivative **4** (0.23 g, 0.53 mmol) and mesylated nitroxide **2** (0.4 g, 1.6 mmol) were stirred in tetrahydrofuran under nitrogen atmosphere and tetra-butyl ammonium fluoride (0.4 g, 1.6 mmol) was added in portions. After 15 min of additional stirring, the mixture was concentrated and chromatographed on silica gel. Compound **5** was eluted by a pentane-ether (8:2) mixture. The product crystallised during concentration of the solvents (0.083 g, 34 %), mp 177-180 °C , elemental analysis calcd for $C_{28}H_{26}N_2O_3Cl$: C, 70.95; H, 5.53; N, 5.91; found: C, 71.17; H, 5.71; N, 5.92; IR (KBr) 3072, 2977, 1628, 1590, 1556, 1515, 1481, 1467, 1452, 1414, 1390, 1353, 1309, 1284, 1215, 1160, 1120, 1072, 1018, 970, 925, 879, 833, 763, 690 and 669 cm^{-1}; MS (FAB(+))*m/z* 474 $(M+1)^+$; UV/Vis (EtOH): λ_{max} (ε) 268 (572030), 336 (4040), 353 (5656), 382 (5494), 404 (5494).

6-chloro-2-[(1-oxyl-2,2,5,5-tetramethyl-pyrrolin-3-yl)methyloxy]-9-propylamino-acridine 6. Labelled phenoxyacridine **5** (0.038 g, 0.08 mmol) was dissolved at 80°C in phenol (5 mL) in the presence of propylamine hydrochloride (0.046 g, 0.48 mmol). After 8 h of stirring, the reaction mixture was cooled to room temperature and poured into diethyl ether (30 mL). The solid was filtered off, washed with diethyl ether. It was then dissolved in a dichloromethane-1N aqueous sodium hydroxide mixture (1/1, 100 mL). The aqueous layer

was extracted three times with dichloromethane The organic layers were combined, washed with 1N aqueous sodium hydroxide, water and brine and finally dried on sodium sulphate. Evaporation of the organic layer gave compound **6** (free base) as a hygroscopic residue (0,24 g, 63 %), IR (KBr) 3600-3000, 3000-2800, 1555, 1450, 1350, 1215, 1155, 1090, 1005, 920, 855, 825, 760, 670 and 650 cm^{-1}; UV/Vis (EtOH): λ_{max} (ε) 286 (32100), 344 (2500), 363 (2150), 424 (7350); HRMS (positive ion FAB, NBA) calcd m/z = 440.2104, found 440.2089 $[M+2H]^+$.

6-Amino-9-[6-chloro-2-((1-oxyl-2,2,5,5-tetramethyl-pyrrolin-3-yl)-methyloxy]-acridine-9-yl]-3,7,11-triazaundecyl-9*H*-purine 8. Labelled phenoxyacridine **5** (0.05 g, 0.1 mmol) was stirred at 80°C in phenol in the presence of the hydrochloride form of the amine **7** (0.27 g, 0.63 mmol). After 2h of stirring, the mixture was poured into 1N aqueous sodium hydroxide (50 mL) and extracted with dichloromethane. The organic layer was washed with water and brine and concentrated *in vacuo*. Compound **8** was precipitated by adding diethyl ether (0.05g, 70 %), mp 115-119 °C, elemental analysis calcd for $C_{35}H_{44}N_{10}O_2Cl$, 1 H_2O: C, 60.90; H, 6.72; N, 20.29; found: C, 60.79; H, 6.75; N, 20.24; IR (KBr) 3600-3000, 3000-2800, 1630, 1590, 1560, 1490-1410, 1350, 1300, 1260-1180, 1160, 1110, 1070, 1020, 925, 825, 800, 760, 720 and 670 cm^{-1}; MS (FAB(+))*m/z* 672 $(M+1)^+$; UV/Vis (EtOH): λ_{max} (ε) 277 (105900), 345 (2000), 362 (2500), 422 (6200).

DNA binding.

DNA binding studies were performed using fluorescence spectrometry and EPR spectroscopy. Solutions were made in 25 mM Tris-HCl, pH 7, 0.1 M NaCl, 0.2 mM EDTA. Compounds **6** and **8** were solubilized in DMSO and then diluted in the buffer, the final concentration of DMSO was 2 %. For fluorescence studies, excitation and emission were monitored at the acridine bands (420, 490 nm respectively). The DNA concentration was 1 10^{-5} M (in base pairs) and that of the tested drugs **6** and **8** varied from 10^{-6} M to 10^{-5} M. For the EPR experiments, we used two ranges of concentrations depending on the nature of the drug. For compound **6**, the DNA concentration was 1 10^{-3} or 5 10^{-4} M (in base pairs) and the drug concentration varied from 1 10^{-5} M to 2 10^{-4} M. For compound **8**, the DNA concentration was 1 10^{-4} M (in base pairs) and the drug concentration varied from 0.5 10^{-5} M to 7 10^{-5} M.

Acknowledgements

We gratefully acknowledge the "Association pour la Recherche sur le Cancer", the "Ligue Nationale Française Contre le Cancer" and the "Ministère de la Recherche et de l'Enseignement Supérieur" for financial support.

Reference and notes.

1 Yamaoka, K.; Noji, S. *Chem. Lett.* **1977**, 449-452; Sinha, B. K.; Chignell, C. F. *Life Sciences* **1976**, *17*, 1829; Sinha, B. K.; Cysyk, R. L.; Millar, D. B.; Chignell, C. F. *J. Med. Chem.* **1976**, *19*, 994

2 Hurley, L.; Osei-Gyimah, P.; Archer, S.; Scholes, C. P.; Lerman, L. S. *Biochemistry* **1982**, *21*, 4999

3 Robinson, B. H.; Lerman, L. S.; Beth, A. H.; Frisch, H. L.; Dalton, L. R.; Auer, C. *J. Mol. Biol.* **1980**, *139*, 19

4 Hong, S.-J.; Piette, L. H. *Cancer Res.* **1976**, *36*, 1159

5 Spielmann, H. P.; Chi, D.-Y.; Hunt, N. G.; Klein, M. P.; Hearst, J. E. *Biochemistry* **1995**, *34*, 14801

6 Carrier, A.; Le Ber, P.; Auclair, C. *Biochemistry* **1990**, *29*, 6002

7 Ottaviani, M. F.; Ghatlia, N. D.; Bossmann, S. H.; Barton, J. K.; Dürr, H.; Turro, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 8946

8 Lemay, P.; Bernier, J.-L.; Henichart, J.-P.; Catteau, J.-P. *Biochem. Biophys. Res. Comm.* **1983**, *111*, 1074; Bailly, C.; Henichart, J. P. *Biochem. Biophys. Res. Comm.* **1990**, *167*, 798

9 Strobel, O. K.; Kryak, D. D.; Bobst, E. V.; Bobst, A. M. *Bioconjugate Chem.* **1991**, *2*, 89

10 *Medicinal Chemistry, Vol.12: Antimalarial Agents: Chemistry and Pharmacology*, Thompson, P. E.; Werbel, M. L., Eds.; Academic Press, New-York, 1972; pp. 123-149

11 For a review-article, see: Thuong, N. T.; Helene, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666

12 Coppel, Y.; Constant, J.-F.; Coulombeau, C.; Demeunynck, M.; Garcia, J.; Lhomme, J.*Biochemistry* **1997**, *36*, 4831

13 Fkyerat, A.; Demeunynck, M.; Constant, J.-F.; Michon, P.; Lhomme, J. *J. Am. Chem. Soc.* **1993**, *115*, 9952

14 Carlson, W. W.; Cretcher, L. H. *J. Am. Chem. Soc.* **1948**, *70*, 597

15 Rosantsev, E. G.; Krinitzkaya, L. A. *Tetrahedron* **1965**, *21*, 491; Hideg, K.; Hankovszky, H. O.; Lex, L. *Synthesis* **1980**, *11*, 911; Hankovszky, H. O.; Hideg, K.; Lex, L. *Synthesis* **1980**, *11*, 914

16 Kivelson, D. *J. Chem. Phys.* **1960**, *33*, 1094

17 Kuznetsov, A. N.; Wasserman, A. M.; Volkov, A. J.; Korst, N. N. *Chem. Phys. Lett.* **1971**, *12*, 103

18 Mc Ghee, J. D.; Von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469