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A transesterification reaction is implicated in the covalent binding of benzo[*b*]acronycine anticancer agents with DNA and glutathion

Marie-Hélène David-Cordonnier,[a] William Laine,[a] Mostafa Kouach,[b] Gilbert Briand,[b] Hervé Vezin,[c] Thomas Gaslonde,[d] Sylvie Michel,[d] Huong Doan Thi Mai,[d] Francois Tillequin,[d] Michel Koch,[d] Stéphane Léonce,[e] Alain Pierré[e] and Christian Bailly[a,*]

[a]*INSERM U-524 et Laboratoire de Pharmacologie Antitumorale du Centre Oscar Lambret, IRCL, Lille 59045, France*
[b]*Laboratoire de Spectrométrie de Masse, Université de Lille 2, Lille 59045, France*
[c]*Laboratoire de Chimie Organique Physique, CNRS UMR8009, USTL Bât. C3, 59655 Villeneuve d'Ascq, France*
[d]*Laboratoire de Pharmacognosie, Université René Descartes (Paris 5), CNRS UMR8638, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75270 Paris cedex 06, France*
[e]*Division Recherche Cancérologie, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy sur Seine, France*

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Abstract—The benzo[*b*]acronycine derivative S23906-1 has been recently identified as a promising antitumor agent, showing remarkable in vivo activities against a panel of solid tumors. The anticancer activity is attributed to the capacity of the drug to alkylate DNA, selectively at the exocyclic 2-amino group of guanine residues. Hydrolysis of the C-1 and C-2 acetate groups of S23906-1 provides the diol compound S28907-1 which is inactive whereas the intermediate C-2 monoacetate derivative S28687-1 is both highly reactive toward DNA and cytotoxic. The reactivity of this later compound S28687-1 toward two bionucleophiles, DNA and the tripeptide glutathion, has been investigated by mass spectrometry to identify the nature of the (type II) covalent adducts characterized by the loss of the acetate group at position 2. On the basis of NMR and molecular modeling analyses, the reaction mechanism is explained by a transesterification process where the acetate leaving group is transferred from position C-2 to C-1. Altogether, the study validates the reaction scheme of benzo[b]acronycine derivative with its target.

1. Introduction

The need for new anticancer agents is pressing. Much effort is currently directed toward the discovery of cytostatic agents targeting the cell-cycle pathway, angiogenesis, or cell differentiation, but conventional cytotoxic agents interfering with DNA metabolism remain actively searched as well.[1] Promising anticancer agents that bind directly to DNA or inhibiting DNA-binding enzymes such as topoisomerases and telomerase have been identified over the past few years.[2] The recent development of small molecules like ecteinascidin 743,[3] brostacillin,[4,5] pyrrolobenzodiazepines,[6,7] CC-1065 and duocarmycin analogues,[8,9] and irofulven[10] clearly indicates a resurgence of interest in DNA alkylating agents. This is also the case for the benzo[*b*]acronycine derivative S23906-1 [(±)-*cis*-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one; Fig. 1], which has revealed promising antitumor activities[11–13] presumably as a result of its capacity to bind covalently to the 2-amino group of guanine residues in DNA.[14] The chemical pathway leading to the formation of S23906-1-guanine adducts is not yet fully elucidated but a very recent structure–activity relationships study demonstrated that the reaction of the drug with DNA involves the acetate group at position 1 as an efficient leaving group.[15] S23906-1 can undergo two types of reactions. Direct interaction with DNA leads to the formation of guanine adducts (hereafter referred to as type I adducts) which have not yet been isolated but nevertheless characterized by fluorescence studies.[14] Similarly, the drug can react with other biological nucleophiles such as the tripeptide glutathion

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* Corresponding author. Tel.: +33-320-169218; fax +33-320-169229; e-mail: bailly@lille.inserm.fr

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(GSH) to form the S-linked drug adducts which may correspond to a cellular detoxification pathway.[16] In both types of adducts, be it with DNA or GSH, the acetate group at position 2 on the pyrano ring of the tetracyclic chromophore is preserved (type I adducts in Fig. 1). Alternatively, S23906-1 can hydrolyze to give the corresponding monoacetate derivative S28687-1 which is considerably more reactive toward DNA and GSH than the parent diacetate drug. A further hydrolysis furnishes the diol compound S23907-1 which does not bind to DNA and is totally inactive in the cytotoxicity assays.[15] We knew that monoacetate derivative S28687-1 rapidly forms covalent complexes with DNA and GSH[14,16] but a more detailed analysis of the reaction products by means of mass spectrometry reveals that this compound never gives type I adducts with DNA or glutathion. All S28687-1 adducts are of type II characterized by the loss of the acetate group at position 2, as depicted in Figure 1. The reaction mechanism proposed for the exclusive formation of type II adducts with S28687-1 implies a transesterification step where the acetate leaving group is transferred from position 2 to position 1 (Fig. 2). This reaction was characterized here by NMR and is consistent with a molecular modeling analysis to rationalize the reaction scheme of S23906-1 with its target. Altogether, the results reported here establish the nucleophilic reactivity of S23906-1 and S28687 toward DNA and glutathion.

2. Results

2.1. Bonding of S28687-1 to DNA

Mass spectrometry was used to characterize the covalent binding of the monoacetate compound to DNA. The 7-bp hairpin oligonuclotide d(CTATGACTCTC GTCATAG)(loop underlined) was incubated with 50 μM S28687-1 in 1 mM ammonium acetate pH 7.15 and the resulting products were analyzed by mass spectrometry with a negative ion mode detection. After a 30 min incubation period (Fig. 3a), the mass spectra show two sets of peaks corresponding to the free oligonucleotide and 1:1 drug–DNA complexes. Peaks at $m/z = 777.3$, 907.1 and 1088.6 refer to the unbound oligonucleotide with the expected mass of 5448 for this sequence. The smaller peaks at $m/z = 832.7$, 971.9 and 1066.1 illustrate the formation of 1:1 drug–DNA covalent adducts for which the DNA-bound drug molecules exclusively bear an OH group at position 2, as depicted in Fig. 3a. After an overnight reaction period (Fig. 3b), the extent of 1:1 drug–DNA complexes is much higher and peaks corresponding to the formation of 2:1 drug–DNA covalent adducts are also visible ($m/z = 888.2$, 1036.3 and 1243.8). Again these peaks coincide with the loss of the acetate group at position 2. All adducts detected are of the type II. We could never identify type I adducts with S28687-1, whatever the experimental conditions used.

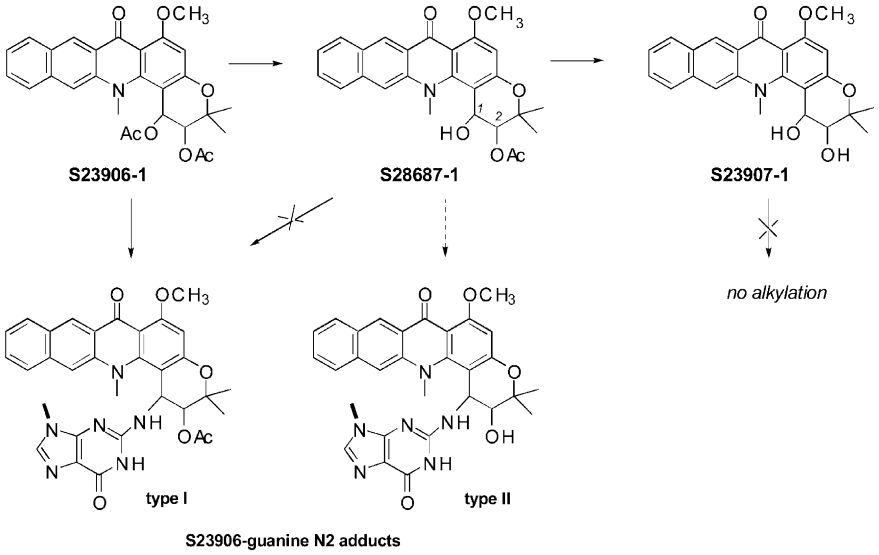

Figure 1. Reaction scheme for S23906-1. Hydrolysis of the diacetate compound (S23906-1) affords the monoacetate (S28687-1) and diol (S23907-1) derivatives. Covalent binding to guanine bases in DNA gives two types of adducts (type I) with an acetate or (type II) a OH group at position 2 on the pyrano ring.

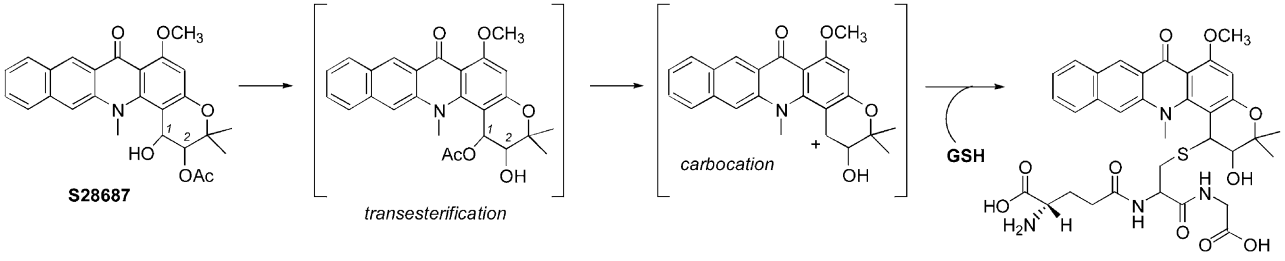

Figure 2. Reaction scheme for the covalent binding of S28687-1 to glutathion. The intermediate transesterification step and the formation of a carbocation are depicted.

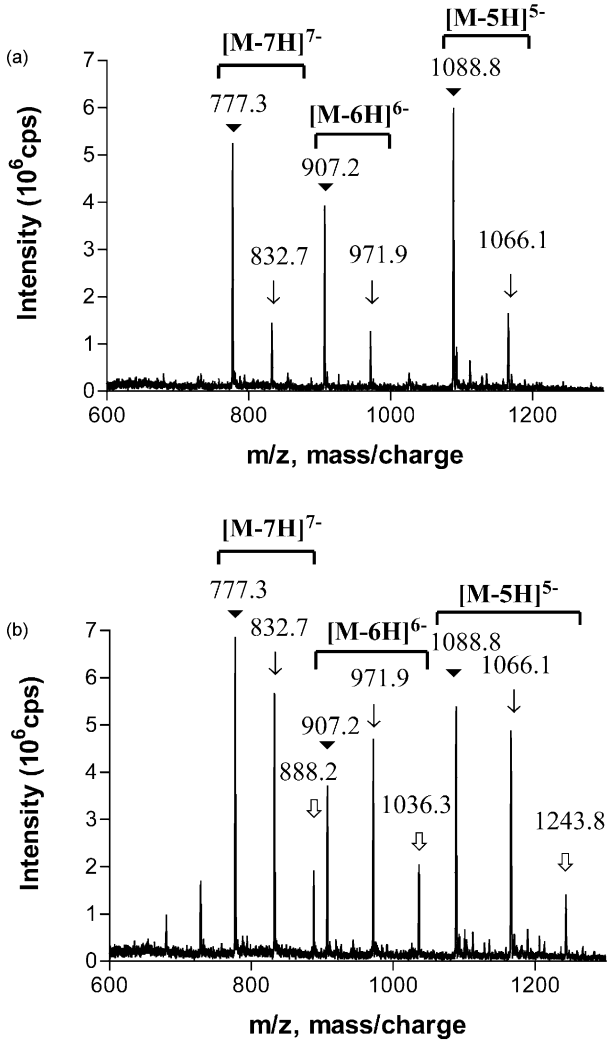

Figure 3. Electrospray ionization mass spectra of the 18-mer hairpin oligonucleotide d(CTATGACTCTCGTCATAG) (duplex underlined) incubated with S28687-1 (A) for 30 min or (B) overnight. Deconvolution of the spectra (negative ion mode) show the presence of three species: (▼) the uncomplexed oligonucleotide (M = 5448), (↓) the 1:1 (M = 5836) and (⇩) 2:1 (M = 6224) drug–DNA covalent complexes.

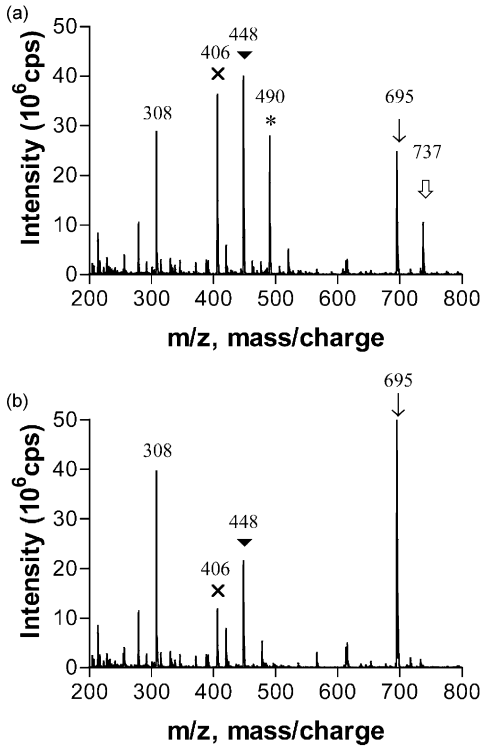

Figure 4. Electrospray ionization mass spectra of S23906-1 (A) or S28687-1 (B) bonding to glutathion. The drugs (100 μM) were incubated with 100 μM GSH (M = 307), for 18 h at 37 °C in 1 mM ammonium acetate pH 7.15 prior to the mass spectrometry measurements (positive ion mode). Among the different species identified for each spectrum, four species present the same molecular weight and correspond to: the free GSH ($MH^+ = 308$), (×) the diol form derived from spontaneous hydrolysis of the drug ($MH^+ = 406$), (▼) the mono-acetate form ($MH^+ = 448$) and (↓) the covalent binding of the drug as a 2-OH form (type II) to GSH ($MH^+ = 695$). Two other peaks are present only in panel A: (∗) the parent S23906-1 di-acetate form ($MH^+ = 490$) and (⇩) the covalent adduct as a 2-acetate form (type I) ($MH^+ = 737$).

2.2. Bonding of S28687-1 to glutathion

The reactivity of the monoacetate compound was investigated further using the tripeptide glutathion (L-γ-glutamyl-L-cysteinyl-glycine) as a model bionucleophile. We compared the nature of the reaction products obtained after incubating S23906-1 or S28687-1 with glutathion for 18 h at 37 °C. Samples were analyzed by EI–MS and typical spectra are shown in Fig. 4. The diacetate compound provides two types of adducts with a mass of 695 and 737 which respectively correspond to the 2-OH and 2-acetate drug-glutathion adducts as depicted in Figure 4. In sharp contrast, compound S28687-1 gives exclusively one type of adducts for which the glutathion-bound molecules all have an OH group at position 2. Here again, as with DNA, we could never detect adducts with an acetate group an position 2 when the reaction was performed with S28687-1.

The two sets of mass spectrometry experiments, with DNA and GSH, lead us to consider that the mono-acetate compound S28687-1 undergoes a transesterification reaction which shift the position of the acetate group from position 2 to position 1 prior to the reaction with the target nucleophile. This hypothesis is fully consistent with the following NMR data.

2.3. NMR study

D_2O (0.1 mL) was added to solution of (±)-*cis*-1-hydroxy-2-acetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro - 7*H* - benzo[*b*]pyrano[3,2 - *h*]acridin - 7 - one (15 mg) in DMSO-d_6 (0.5 mL). The ^{1}H NMR spectrum was recorded immediately and additional spectra were recorded at intervals over a period of 72 h when the sample was kept at 20 °C (Fig. 5). With time, characteristic modifications were noticed, including: (i) the appearance of an additional singlet corresponding to the acetate group at C-1 at 1.95 ppm, and (ii) the appearance of two doublets ($J = 1.5$ Hz) at 4.05 and 6.15 ppm, corresponding to the signals of H-2 and H-1 of (±)-*cis*-1-acetoxy-2-hydroxy-6-methoxy-3,3,14-trimethyl - 1,2,3,14 - tetrahydro - 7*H* - benzo[*b*]pyrano[3,2 - *h*]acridin-7-one, respectively. After 72 h incubation, the

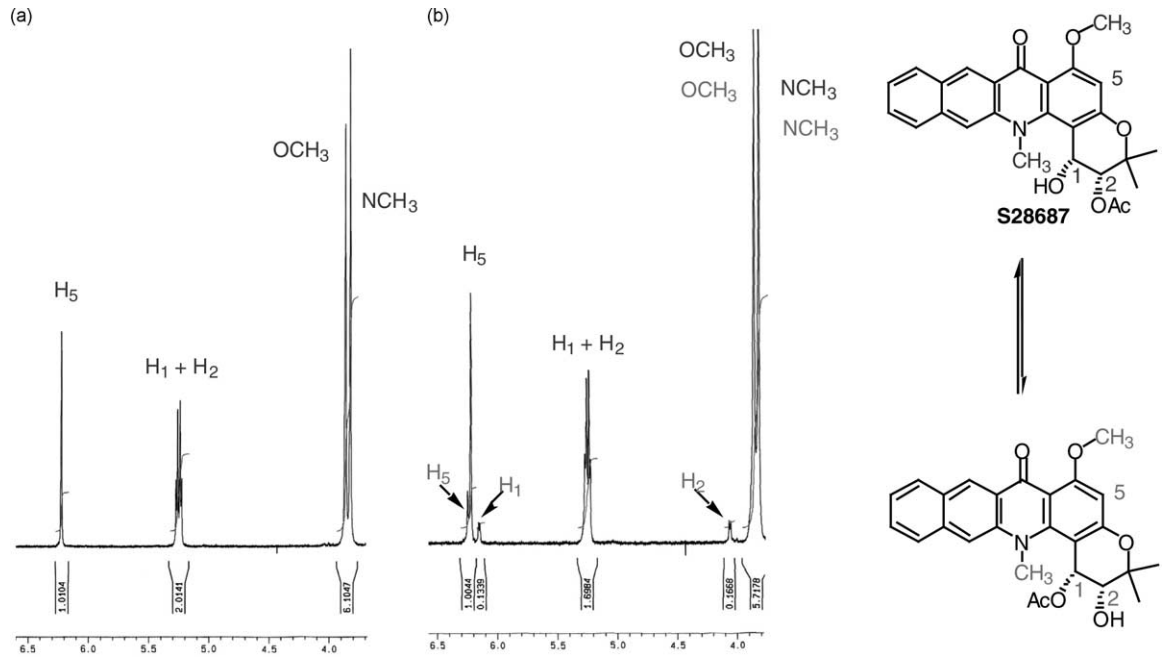

Figure 5. ^{1}H NMR study of S28687-1 transesterification in DMSO-d_6/D_2O solution at 20 °C. Spectra are recorded at (a) $t = 0$ and (b) $t = 24$ h.

equilibrium was reached and the resulting solution contained 19% of the C1-monoacetate compound.

2.4. Molecular modeling

A Monte Carlo analysis performed with the SS_*cis* diasteroisomer of S23906-1 gave eight conformers of similar global minimum energy. The conformers only differ by the relative orientations of the acetate and methoxy groups, the main core of the molecule being essentially rigid. The resulting structure was optimized at the DFT B3LYP/6-31G** level for the ground state and the carbocation species (Fig. 6a). Calculation of the transition state leading to the departure of the acetate at position 1 gives an imaginary frequency corresponding to the elongation of the C–O acetate chemical bond. The transition between the initial structure and this transition state conformation demands an energy gain of +51 kcal/mol. The next step corresponding to the formation of the carbocation species at position 1 requires a formation energy of +101 kcal/mol. A molecular orbital analysis performed with this carbocation indicates that the energy of the lowest unoccupied molecular orbital (E_{LUMO}) is mainly localized on carbon 1 indicating a strong electrophilic reactivity at this site. The C1 position of S23906-1 thus appears as a zone of the molecule where electron interaction is most likely to occur.

The alternative configuration with the carbocation at position 2 was also calculated but it is considered impossible for two reasons. First, the C2-carbocation requires a 21 kcal/mol higher energy than the C1-carbocation. Second, the LUMO energy remains mainly distributed on the C1 position. The C2-position is chemically inert. Moreover, an optimization of the C2-carbocation structure at the DFT level showed a translocation of the acetate from position 1 to position 2 (data not shown). The modeling study leaves no room for doubt that the most favorable structure corresponds to the C1-carbocation.

As regards the monoacetate derivative, the energetic analysis shows very little difference between the experimental drug S28687-1 having the acetate at position 2 and the analogous virtual drug with the acetate at position 1 (Fig. 6b). The energy barrier between the two conformations is minimal ($\Delta_{\Delta Hf} = -1$ kcal/mol), consistent with a facile *trans*-esterification. The energy required for the formation of the C1-carbocation is +89 kcal/mol, as opposed to +152 kcal/mol with the diacetate. This theoretical observation agrees with our previous experimental results showing that the monoacetate derivative S28687-1 reacts more strongly with DNA than the diacetate S23906-1.[14]

3. Discussion

The plant alkaloid acronycine, initially isolated from the bark of *Acronychia baueri* (also known as *Sarcomelicope simplicifolia*),[17,18] is probably a natural toxin produced by the tree (a Rutaceous) to combat herbivores and/or microorganisms. Our goal is to convert this plant toxin into a useful therapeutic agent for the treatment of cancer. Acronycine displays antitumor activities in vivo[19] but the use of this tetracyclic compound in cancer chemothrerapy was unsuccessful. Clinical trials initiated in the mid-1970s showed insufficient response and the development of acronycine was arrested in the early 1980s. The exact mechanism of action of this natural product remains obscure. Acronycine does not bind to DNA but it inhibits DNA synthesis in tumor cells.[20,21] It is suspected that acronycine is bioactivated in cells into a DNA-reactive intermediate.[22] The most likely metabolite is acronycine epoxide (Fig. 7) which has been

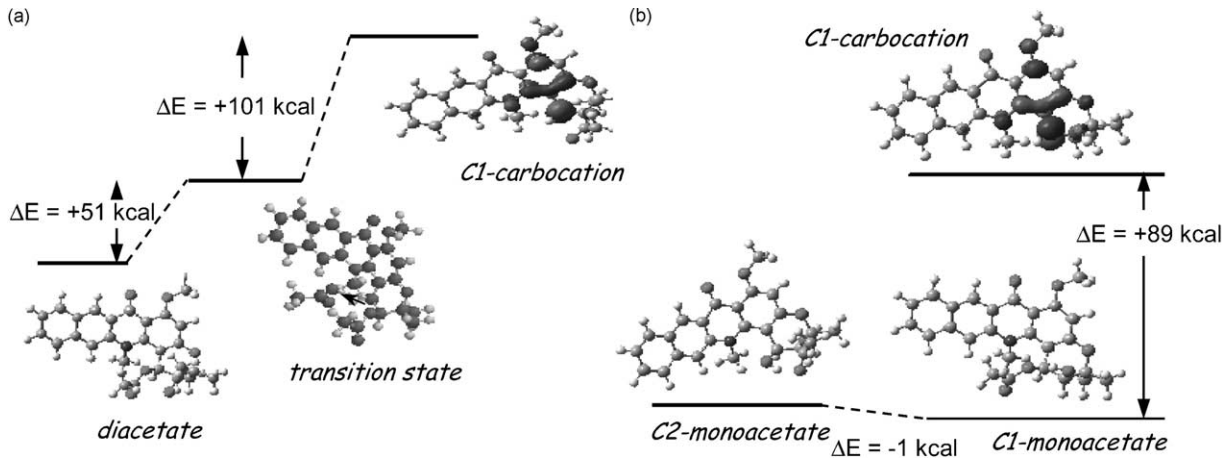

Figure 6. (a) Structural and energetic analyses of the SS_*cis* diasteroisomer of S23906-1. The diagram indicates the energy barriers between the diacetate compound, the transition state intermediate and the C1-carbocation. The LUMO distribution for the most stable calculated configuration of the C1-carbocation is indicated. (b) Conformational and molecular orbital analyses of the C2-monoacetate compound S28687-1 and the analogous C1-monoacetate derivative. The energy required for the formation of the C1-carbocation is indicated.

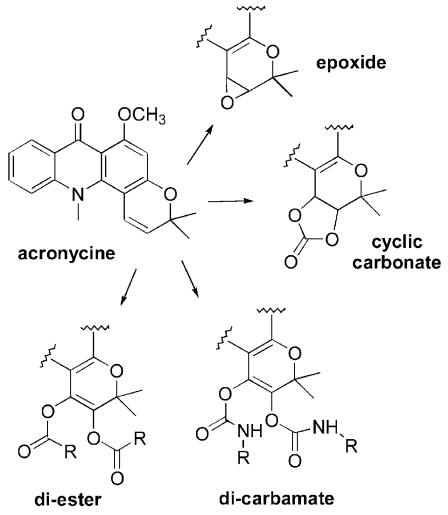

Figure 7. Structure of acronycine and derivatives.

isolated in small quantities during the purification of acronycine from the plant.[23] The epoxide derivative may well represent the bioactived form of acronycine responsible for DNA alkylation. This hypothesis is all the more plausible that epoxide functions are frequently encountered in DNA reactive drugs, such as the pluramycins,[24–26] azinomycins[27,28] and clerocidins[29,30] for examples.

Over the past 10 years, a variety of acronycine derivatives have been synthesized.[15,22,31–37] Two important observations have emerged from the structure–activity relationship studies. First, the convertion of the tetracyclic chromophore into a pentacyclic system reinforces antitumor activity. Benzo[*b*]acronycine derivatives usually present higher cytotoxic potential than the corresponding acronycine derivatives. The gain of cytotoxicity conferred by the additional benzo ring is not yet fully understood but the presence of a larger planar chromophore certainly reinforces stacking interaction with DNA bases. Second, a significant gain of cytotoxicity was observed when introducing ester functions at positions 1 and 2 on the pyrano ring. The diacetate compound S23906-1 was selected for a preclinical development but other bisubstituted analogues (e.g., dicarbamates) also showed interesting cytotoxicity profiles. The most cytotoxic compounds in the series are the cyclic carbonate derivatives (Fig. 7) which are tremendously toxic to tumor cells in vitro.[35] Unfortunately, they are also toxic to animals and do not show better in vivo antitumor activity compared to S23906-1. The in vitro cytotoxic potential of the diacetate compound is not very potent, the IC_{50}s being in general in the 0.1–1 μM range. However, S23906-1 is 5–10-fold more potent than cisplatin.

Benzo[*b*]acronycine derivatives highly reactive toward DNA, such as the dicarbamates, are generally too toxic and the safety margin is too weak for use in vivo. On the opposite, unreactive benzo[*b*]acronycine derivatives, such as the diol S23907-1 or the 1-keto derivatives, are devoid of cytotoxicity and show no antitumor activity. We have now good evidences that DNA alkylation is necessary for antitumor activity in this series.[15] The diacetate derivative S23906-1 optimally combines reactivity and stability. It is sufficiently activated for covalent reaction with DNA but its intrinsic reactivity is sufficiently moderated so that the compound is not trapped by other bionucleophiles before reaching its primary target in the cell nuclei. With S23906-1, the DNA alkylation potential is judiciously controlled. This compound can be considered both as a drug and a prodrug because it is active by itself, generating type I adducts with DNA, and further activated by hydrolysis, giving type II adducts.

We have previously demonstrated that S23906-1 form covalent complexes with DNA. The exocyclic 2-amino group of guanines exposed in the minor groove of double helical DNA is the reactive site.[14] Very recently, we found that this drug can also react with the tripeptide glutathion which probably serves as a detoxification system in cells.[16] In both cases, with DNA and GSH, we identified type I adducts characterized by the presence

of an acetate group at position 2 on the pyrano ring. Parallel experiments performed with the monoacetate analogue S28687-1 afford a different type of adducts, type II, characterized by a OH group at position 2. The present study provides a sound explanation for the formation of the two slightly different types of adducts. A transesterification reaction, directly observed by NMR with the free drug, is responsible for the shift of the acetate from position 2 to position 1. The modeling study affords an interesting theoretical support for the transesterification reaction which accounts satisfactorily for the production of type II adducts. The computer study also serves to identify a potential reactive intermediate, the C1-carbocation. This highly reactive cationic species can be generated easily from the monoacetate S28687-1 and to a lower extent from the diacetate S23906-1.

We have now acquired a solid knowledge of the chemical reactivity of S23906-1 and this molecular basis can be use to generate superior analogues and/or to better control the reactivity of the parent compound. The present study further attests that benzo[*b*]acronycines represent a very interesting series of molecules, both from the therapeutic as promising anticancer agents for the treatment of cancers and also from the basic points of view to dissect the mechanism of DNA recognition and alkylation by small molecules.

4. Experimental

4.1. Drugs

The synthesis of the benzo[*b*]acronycine derivatives S23906-1, S28687-1 and the diol S23907-1 has been reported previously.[35]

4.2. Electrospray ionisation mass spectroscopy (EI-MS)

The alkylation of the 18-mer oligonucleotide by S28687-1 was monitored by EI-MS. Both drug and DNA (50 μM each) were incubated for 30 min or overnight in 200 μL of 1 mM ammonium acetate, pH 7.15 prior to be injected in a simple-quadrupole mass spectrometer API I (Perkin-Elmer Sciex) equipped with an ion-spray (nebulizer-assisted electrospray) source (Sciex, Toronto, Canada) using a needle pre-washed with methanol. The solutions were continuously infused with a medical infusion pump (Model 11, Harvard Apparatus, South Natick, USA) at a flow rate of 5 μL/min. Polypropylene glycol was used to calibrate the quadrupole. Ion spray mass spectra were acquired at unit resolution by scanning from m/z 600 to 1300 with a step size of 0.1 Da and a dwell time of 2 ms. Twenty spectra were summed and recorded at an orifice voltage of −60 V whereas the potential of spray needle was held at −4.5 kV.

To evidence the bonding of S28687-1 and S23906-1 to glutathion by EI–MS, 100 μM of glutathion was mixed to 100 μM of S23906-1 or S28687-1 compounds for 18 h at 37 °C in 200 μL of 1 mM ammonium acetate, pH 7.15. The samples were then injected as previously described and the ion spray mass spectra were acquired at unit resolution by scanning from m/z 200–800 with a step size of 0.1 Da and a dwell time of 2 ms. Twenty spectra were summed and recorded at an orifice voltage of +50 V whereas the potential of spray needle was held at +5 kV.

4.3. NMR study

^{1}H NMR spectra were recorded at 400 MHz using a Bruker AVANCE 400 spectrometer.

4.4. Computational chemistry

All calculations were performed on a NT workstation (PIV-2.4 GHz processor) using the Spartan Pro V 1.0.1 and Gaussian 98 package softwares. A conformational analysis was performed for the SS_*cis* compound on all rotatable bonds using Monte Carlo implemented in Spartan and then the resulting structures were minimized using MMF94 force field and fully optimized at the DFT level using hydrid B3LYP functional density with 6-31G** basis set. The transition state calculations were also performed with the Spartan package.

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References and notes

1. Anthoney, D. A.; Twelves, C. J. *Am. J. Pharmacogenomics* **2001**, *1*, 67.
2. Demeunynck, M., Bailly, C., Wilson, W. D. *Small Molecule DNA and RNA Binders*; Darmstadt, Wiley-VCH: 2003.
3. Aune, G. J.; Furuta, T.; Pommier, Y. *Anticancer Drugs* **2002**, *13*, 545.
4. Cozzi, P. *Farmaco* **2001**, *56*, 57.
5. Geroni, C.; Marchini, S.; Cozzi, P.; Galliera, E.; Ragg, E.; Colombo, T.; Battaglia, R.; Howard, M.; D'Incalci, M.; Broggini, M. *Cancer Res.* **2002**, *62*, 2332.
6. Wilson, S. C.; Howard, P. W.; Forrow, S. M.; Hartley, J. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* **1999**, *42*, 4028.
7. Gregson, S. J.; Howard, P. W.; Hartley, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* **2001**, *44*, 737.
8. Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1438.
9. Smith, J. A.; Bifulco, G.; Case, D. A.; Boger, D. L.; Gomez-Paloma, L.; Chazin, W. J. *J. Mol. Biol.* **2000**, *300*, 1195.
10. Wolkenberg, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477.
11. Léonce, S.; Perez, V.; Lambel, S.; Peyroulan, D.; Tillequin, F.; Michel, S.; Koch, M.; Pfeiffer, B.; Atassi, G.; Hickman, J. A.; Pierré, A. *Mol. Pharmacol.* **2001**, *60*, 1383.
12. Guilbaud, N.; Kraus-Berthier, L.; Meyer-Losic, F.;

Malivet, V.; Chacun, C.; Jan, M.; Tillequin, F.; Koch, M.; Pfeiffer, B.; Atassi, G.; Hickman, J.; Pierré, A. *Clin. Cancer Res.* **2001**, *7*, 2573.
13. Guilbaud, N.; Léonce, S.; Tillequin, F.; Koch, M.; Hickman, J. A.; Pierré, A. *Anticancer Drugs* **2002**, *13*, 445.
14. David-Cordonnier, M.-H.; Laine, W.; Lansiaux, A.; Kouach, M.; Briand, G.; Pierré, A.; Hickman, J. A.; Bailly, C. *Biochemistry* **2002**, *41*, 9911.
15. Doan Thi Mai, H.; Gaslonde, T.; Michel, S.; Tillequin, F.; Koch, M.; Bongui, J.-B.; Elomri, A.; Seguin, E.; Pfeiffer, B.; Renard, P.; David-Cordonnier, M.-H.; Tardy, C.; Laine, W.; Bailly, C.; Kraus-Berthier, L.; Léonce, S.; Hickman, J. A.; Pierré, A. *J. Med. Chem.* **2003**, *46*, 3072.
16. David-Cordonnier, M.-H.; Laine, W.; Joubert, A.; Tardy, C.; Goossens, J. F.; Kouach, M.; Briand, G.; Doan Thi Mai, H.; Michel, S.; Tillequin, F.; Koch, M.; Léonce, S.; Pierré, A.; Bailly, C. *Eur. J. Biochem.* **2003**, *270*, 2848.
17. Hughes, G. K.; Lahey, F. N.; Price, J. R.; Webb, L. J. *Nature* **1948**, *162*, 223.
18. Svoboda, G.; Poore, G. A.; Simpson, P. J.; Boder, G. B. *J. Pharm. Sci.* **1966**, *55*, 759.
19. Dorr, R. T.; Liddil, J. D.; Von Hoff, D. D.; Soble, M.; Osborne, C. K. *Cancer Res.* **1989**, *49*, 340.
20. Dunn, B. P.; Gout, P. W.; Beer, C. T. *Cancer Res.* **1973**, *33*, 2310.
21. Gout, P. W.; Dunn, B. P.; Beer, C. T. *J. Cell. Physiol.* **1971**, *78*, 127.
22. Tillequin, F. *Ann. Pharm. Fr.* **2002**, *60*, 246.
23. Brum-Bousquet, M.; Mitaku, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M. *Planta Med.* **1988**, *54*, 470.
24. Sun, D.; Hansen, M.; Hurley, L. H. *J. Am. Chem. Soc.* **1995**, *117*, 2430.
25. Hansen, M.; Hurley, L. *Acc. Chem. Res.* **1996**, *29*, 249.
26. Owen, E. A.; Burley, G. A.; Carver, J. A.; Wickham, G.; Keniry, M. A. *Biochem. Biophys. Res. Commun.* **2002**, *290*, 1602.
27. Zang, H.; Gates, K. S. *Biochemistry* **2000**, *39*, 14968.
28. Alcaro, S.; Ortuso, F.; Coleman, R. S. *J. Med. Chem.* **2002**, *45*, 861.
29. Binaschi, M.; Zagotto, G.; Palumbo, M.; Zunino, F.; Farinosi, R.; Capranico, G. *Cancer Res.* **1997**, *57*, 1710.
30. Gatto, B.; Richter, S.; Moro, S.; Capranico, G.; Palumbo, M. *Nucleic Acids Res.* **2001**, *29*, 4224.
31. Shieh, H.-L.; Pezzuto, J. M.; Cordell, G. A. *Chem.-Biol. Interact.* **1992**, *81*, 35.
32. Elomri, A.; Mitaku, S.; Michel, S.; Skaltsounis, A.-L.; Tillequin, F.; Koch, M.; Pierré, A.; Guilbaud, N.; Léonce, S.; Kraus-Berthier, L.; Rolland, Y.; Atassi, G. *J. Med. Chem.* **1996**, *39*, 4762.
33. Kolokythas, G.; Kostakis, I. K.; Pouli, N.; Marakos, P.; Skaltsounis, A. L.; Pratsinis, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1443.
34. Kostakis, I. K.; Magiatis, P.; Pouli, N.; Marakos, P.; Skaltsounis, A. L.; Pratsinis, H.; Léonce, S.; Pierré, A. *J. Med. Chem.* **2002**, *45*, 2599.
35. Costes, N.; Le Deit, H.; Michel, S.; Tillequin, F.; Koch, M.; Pfeiffer, B.; Renard, P.; Léonce, S.; Guilbaud, N.; Kraus-Berthier, L.; Atassi, G.; Pierré, A. *J. Med. Chem.* **2000**, *43*, 2395.
36. Costes, N.; Elomri, A.; Dufat, H.; Michel, S.; Seguin, E.; Koch, M.; Tillequin, F.; Pfeiffer, B.; Renard, P.; Léonce, S.; Pierré, A. *Oncol. Res.* **2003**, *13*, 191.
37. Michel, S.; Seguin, E.; Tillequin, F. *Curr. Med. Chem.* **2002**, *9*, 1689.