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Design, Synthesis and Pharmacological Evaluation of New Anticancer Fused Pentacycles

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The quinoline chromophore has long formed the basis for the clinical development of novel antitumour agents. Camptothecin derivatives have already proved their clinical efficacy and compounds such as ascididemin (pyridoacridine family), DHDMC (protoberberine family) have a very promising future. During our search for new cytotoxic molecules, we have designed compounds based on the benzo[c]pyrido[2,3,4-kl]acridine skeleton which combines the structural features of ascididemin and DHDMC. Corresponding compounds were synthesized and evaluated for their cytotoxic activity against human prostatic PC-3 cell lines. Some have shown promising biological activity in inhibiting the growth of cell lines which are resistant to camptothecin.

INTRODUCTION

The search for new pharmaceuticals from the marine environment has resulted in the isolation of an ever-increasing number of alkaloids based on the pyrido[2,3,4-kl]acridine skeleton.[1] Among them, ascididemin (ASC) (Chart 1) is a planar pentacyclic aromatic DNA-intercalating agent isolated from *Cystodytes dellechiajei*[2] which exerts highly cytotoxic properties towards human colon (HCT 116), breast (MCF 7) cancer cell lines[3] and towards human leukemic cell lines.[2] Plants have also proved to be a very rich source of alkaloids such as protoberberines. These compounds include several naturally occurring and synthetic agents possessing significant and specific pharmacological activity,[4,5] such as 5,6-dihydro-8-desmethylcoralyne (DHDMC) (Chart 1), a potent inducer of topoisomerase I-mediated DNA breaks.[6]

These considerations prompted us to design a new series of potential cytotoxic agents related to the benzo[c]pyrido[2,3,4-kl]acridine skeleton—combining the structural features of ASC and DHDMC (Chart 2). The isoquinoline skeleton of DHDMC was replaced by the quinoline heterocycle present in ASC and one ring bore an extended side chain.

The synthesis of benzopyridoacridines **4–6** is described. Their cytotoxicity was determined on human prostatic PC-3 cell lines.

MATERIALS AND METHODS

Chemistry

Melting points were determined with a Büchi 535 capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Bruker Vector 22. ^{1}H NMR spectra were recorded at 300 MHz, on a Bruker AC 300P machine. Chemical shifts were reported in ppm downfield from TMS as internal standard and J values in Hz. Mass spectra were recorded on a Finnigan Mat SSQ710 mass spectrometer. Elemental analyses were performed by the Service Central d'Analyse-Département Analyse Elémentaire, CNRS, F-69390 Vernaison. Dimethylformamide was distilled from CaH_2 and stored over 3 Å molecular sieves.

3-Acetyl-10-hydroxy-4,5-dimethoxy-2,3,12,13-tetrahydro-1H-benzo[c]pyrido[2,3,4-kl]acridine (4d)

A solution of starting material **1** (1.89 mmol), PPTS (2.84 mmol) and 6-hydroxytetralone (5.67 mmol) in

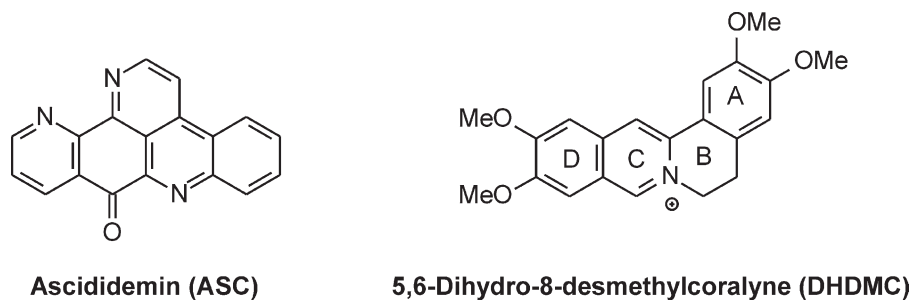**Ascididemin (ASC)**   **5,6-Dihydro-8-desmethylcoralyne (DHDMC)**

CHART 1   Structures of ASC and DHDMC.

butan-1-ol (10 mL) was heated under reflux using a Dean–Stark trap. After cooling, the solvent was evaporated and the residue was triturated with diethyl ether. After washing the precipitate with diethyl ether, the crude product was subjected to chromatography (silica gel, 2% methanol-dichloromethane-TFA (5 drops)). Evaporation of the solvents yielded 78% of **4d** as a yellow solid: mp > 250°C (iPrOH/Et$_2$O); IR (KBr) 1647, 3230 cm^{-1}; ^{1}H NMR (DMSO-d$_6$) δ 2.00 (s, 3H), 2.85–3.01 (m, 6H), 3.36 (sl, 1H), 3.75 (s, 3H), 3.98 (s, 3H), 4.79 (sl, 1H), 6.69 (d, J$_m$ = 1.7, 1H), 6.77 (dd, J$_m$ = 2.1, J$_o$ = 8.6, 1H), 7.29 (s, 1H), 8.22 (d, J$_o$ = 8.6, 1H), 9.79 (s, 1H).

10-Hydroxy-4,5-dimethoxy-3-[2-(4-methyl) piperazin-1-ylacetyl]-2,3,12,13-tetrahydro-1H-benzo[c]pyrido[2,3,4-kl]acridine (6d)

A solution of starting material **2** (1.38 mmol), PPTS (2.07 mmol) and 6-hydroxytetralone (4.14 mmol) in butan-1-ol was heated under reflux using a Dean–Stark trap. The mixture was cooled to room temperature, made basic with 10% aqueous potassium carbonate, diluted with ethyl acetate and the layers separated. The dark red organic layer was washed with saturated aqueous sodium chloride and dried over magnesium sulfate. Removal of the solvent gave an oily product which precipitated in diethyl ether. After filtration and washing with diethyl ether, the crude product was subjected to chromatography (silica gel, 5% methanol-dichloromethane). Evaporation of the solvents yielded 65% of **6d** as a yellow

solid: mp > 234°C (EtOH/Et$_2$O); IR (KBr) 1636, 1659, 3230 cm^{-1}; ^{1}H NMR (DMSO-d$_6$/60°C) δ 2.75 (s, 3H), 2.97–3.30 (m, 14H), 3.86 (s, 4H), 4.06 (s, 7H), 6.89 (d, J$_m$ = 2.2, 1H), 6.94 (dd, J$_m$ = 2.2, J$_o$ = 8.4, 1H), 8.24 (s, 1H), 8.63 (d, J$_o$ = 8.4, 1H).

General Procedure for the Preparation of Benzopyridoacridines 4b,c

Potassium carbonate (3.84 mmol) was added to a solution of the 10-hydroxy derivative **4d** (1.28 mmol) in dry DMF (5 mL) and the mixture was stirred for 30 min at room temperature. The appropriate N-protected chloroalkylamine (1.92 mmol) was then added. The mixture was stirred for 3 h at 80°C, then diluted with water and extracted several times with ethyl acetate. The organic layers were washed with saturated aqueous sodium chloride and dried over magnesium sulfate. Concentration followed by chromatography (silica gel, 2% methanol-dichloromethane) gave a crude pink powder which was dissolved in 10 ml of methanol with Pd-C (10% w/w). The suspension was refluxed for 30 min and ammonium formate (10 eq) was added. The mixture was refluxed for 3 h. The cooled mixture was filtered over Celite, washed with methanol and the solvent evaporated. The crude product was subjected to chromatography (silica gel, 10% methanol-dichloromethane). After evaporation of the solvents, the residue was dissolved in methanol and methanol saturated with HCl was added to yield **4b** or **4c** as hydrochloride.

3-Acetyl-10-(2-aminopropoxy)-4,5-dimethoxy-2,3,12,13-tetrahydro-1H-benzo[c] Pyrido[2,3,4-kl]-acridine Hydrochloride (4b)

    Yield 41%; yellow solid: mp > 250°C (EtOH/Et$_2$O); IR (KBr) 1632, 1678, 3421 cm^{-1}; ^{1}H NMR (DMSO-d$_6$) δ 2.05–2.07 (m, 5H), 2.89–3.01 (m, 6H), 3.20 (sl, 2H), 3.39 (sl, 1H), 3.81 (s, 3H), 4.02 (s, 3H), 4.22 (t, 2H), 4.67 (sl, 1H), 7.08–7.11 (m, 2H), 8.20 (s, 1H), 8.67 (sl, 1H), 9.17 (sl, 3H); MS (EI) m/e 447 (M$^+$, 100), 405 (18), 390 (100), 333 (58), 290 (10); Anal. Calcd for C$_{25}$H$_{29}$N$_3$O$_4$·2HCl (2.5H$_2$O): C, 55.22; H, 6.42; N, 7.43; Cl, 12.54. Found: C, 55.79; H, 6.21; N, 7.61; Cl, 12.18%.
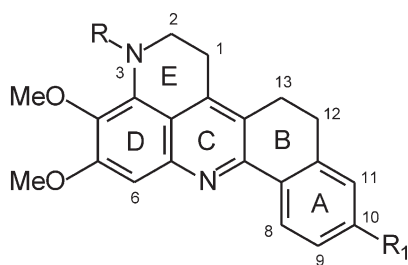
4-6

CHART 2   Benzopyridoacridines.

3-Acetyl-10-(2-aminoethoxy)-4,5-dimethoxy-2,3,12,13-tetrahydro-1*H*-benzo[*c*]Pyrido[2,3,4-*kl*]-acridine Hydrochloride (**4c**)

Yield 35%; yellow solid: mp > 250°C (EtOH/Et$_2$O); IR (KBr) 1633, 1681, 3425 cm^{-1}; ^{1}H NMR (DMSO-d$_6$) δ 2.06 (s, 3H), 2.98–3.27 (m, 8H), 3.56 (sl, 1H), 3.81 (s, 3H), 4.02 (s, 3H), 4.34 (t, 2H), 4.68 (sl, 1H), 7.09–7.12 (m, 2H), 8.09 (sl, 3H), 8.40 (s, 1H), 8.68 (sl, 1H); MS (EI) m/e 433 (M$^+$, 100), 390 (30), 376 (60), 333 (73), 290 (9); Anal. Calcd for C$_{24}$H$_{27}$N$_3$O$_4$·1.75HCl (3H$_2$O): C, 54.46; H, 6.35; N, 7.62; Cl, 11.25. Found: C, 54.38; H, 6.05; N, 7.73; Cl, 11.64%.

General Procedure for the Preparation of Benzopyridoacridines 6b,c

Potassium carbonate (1.2 mmol) was added to a solution of the 10-hydroxy derivative **6d** (0.4 mmol) in dry DMF (5 mL) and the mixture was stirred for 30 min at room temperature. The appropriate N-protected chloroalkylamine (0.6 mmol) was then added. The mixture was stirred for 3.5 h at 80°C, then diluted with water and extracted several times with ethyl acetate. The organic layers were washed with saturated aqueous sodium chloride and dried over magnesium sulfate. Concentration followed by chromatography (silica gel, 20% methanol-dichloromethane) gave a crude white powder which was dissolved in 10 ml of methanol saturated with HCl and the solution stirred at room temperature overnight. Evaporation of the solvent yielded **6b** or **6c** as hydrochloride.

10-(3-Aminopropoxy)-4,5-dimethoxy-3-[2-(4-methyl)piperazin-1-ylacetyl]-2,3,12,13-tetrahydro-1*H*-benzo[*c*]pyrido[2,3,4-*kl*]acridine Hydrochloride (**6b**)

Yield 75%; yellow solid: mp > 250°C (EtOH/Et$_2$O/H$_2$O); IR (KBr) 1633, 1690, 3405 cm^{-1}; ^{1}H NMR (DMSO-d$_6$/60°C) δ 2.10 (qt, J = 6.8, 2H), 2.74 (s, 3H), 3.00–3.24 (m, 16H), 3.85–4.05 (m, 10H), 4.23 (t, J = 6.3, 2H), 7.03 (s, 1H), 7.08 (dd, J$_m$ = 2.7, J$_o$ = 7.2, 1H), 7.90 (s, 1H), 8.11 (sl, 3H), 8.60 (d, J$_o$ = 7.2, 1H); MS (EI) m/e 545 (M$^+$, <4), 513 (<4), 489 (<4), 181 (6),

113 (100), 70 (36); Anal. Calcd for C$_{31}$H$_{39}$N$_5$O$_4$·4HCl (4.5H$_2$O): C, 48.19; H, 6.78; N, 9.06; Cl, 18.35. Found: C, 48.04; H, 6.45; N, 9.01; Cl, 18.80%.

10-(2-Aminoethoxy)-4,5-dimethoxy-3-[2-(4-methyl)piperazin-1-ylacetyl]-2,3,12,13-tetrahydro-1*H*-benzo[*c*]pyrido[2,3,4-*kl*]acridine Hydrochloride (**6c**)

Yield 65%; yellow solid: mp > 250°C (EtOH/Et$_2$O/H$_2$O); IR (KBr) 1631, 1667, 2934 cm^{-1}; ^{1}H NMR (DMSO-d$_6$/60°C) δ 2.74 (s, 3H), 3.02–3.27 (m, 16H), 3.86 (s, 3H), 4.06 (s, 6H), 4.38 (tl, J = 5.2, 3H), 7.08 (s, 1H), 7.10 (dd, J$_m$ = 2.2, J$_o$ = 8.8, 1H), 8.04 (s, 1H), 8.39 (sl, 3H), 8.68 (d, J = 8.8, 1H); MS (EI) m/e 531 (M$^+$, 5), 499 (8), 377 (9), 334 (12), 113 (100), 70 (72); Anal. Calcd for C$_{30}$H$_{37}$N$_5$O$_4$·4HCl (9H$_2$O): C, 42.91; H, 7.08; N, 8.34; Cl, 16.88. Found: C, 43.00; H, 6.99; N, 8.45; Cl, 16.85%.

General Procedure for the Preparation of Benzopyridoacridines 5a–c

A solution of compounds **4a–c** in aqueous hydrochloric acid 6 N was refluxed for 4 h. The cooled mixture was made alkaline with potassium bicarbonate and partitioned between ethyl acetate and water. The organic layer was washed with water and saturated aqueous sodium chloride and dried over magnesium sulfate. Concentration was followed by chromatography (silica gel, 10% methanol–1% ammoniacal dichloromethane). After evaporation of the solvents, the residue was dissolved firstly in methanol and then methanol saturated with HCl was added to yield **5a**, **5b** or **5c** as hydrochloride.

4,5-Dimethoxy-10-[3-(4-methyl)piperazin-1-ylpropoxy]-2,3,12,13-tetrahydro-1*H*-benzo[*c*]pyrido-[2,3,4-*kl*]acridine Hydrochloride (**5a**)

Yield 30%; red solid: mp > 250°C (EtOH/Et$_2$O); IR (KBr) 1645, 3376 cm^{-1}; ^{1}H NMR (DMSO-d$_6$) δ 2.27 (sl, 2H), 2.84 (s, 3H), 2.93–2.99 (m, 4H), 3.22–3.70 (m, 14H), 3.75 (s, 3H), 3.97 (s, 6H), 4.25 (t, J = 5.6, 2H), 6.82 (sl, 2H), 7.12 (s, 1H), 7.13 (d, J$_o$ = 10.1, 1H), 7.49 (s, 1H), 8.55 (d, J = 8.3, 1H); MS (EI) m/e 348
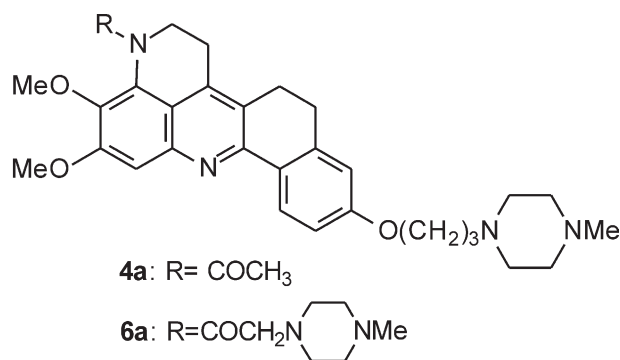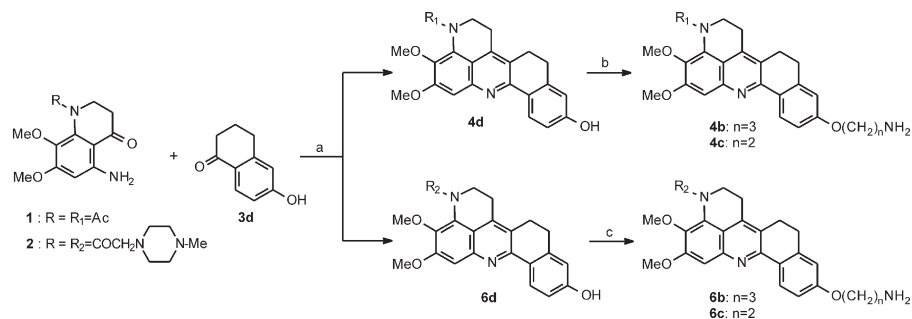

4a: R= COCH$_3$

6a: R=COCH$_2$N⟨ ⟩NMe

CHART 3 Structure of pentacycles **4a** and **6a**.

SCHEME 1 Conditions and reagents: (a) PPTS, butan-1-o1, reflux; (b) i: Cl(CH₂)ₙNHCbz, K₂CO₃, DMF, 80°C; ii: Pd on C, HCOONH₄, MeOH, reflux; (c) i: Cl(CH₂)ₙNHBoc, K₂CO₃, DMF, 80°C; ii: MeOH, HCl, rt.

(M$^+$, 64), 333 (100), 290 (8), 166 (22); Anal. Calcd for $C_{29}H_{36}N_4O_3 \cdot 4HCl$ (4H₂O): C, 52.06; H, 6.93; N, 8.37; Cl, 15.89. Found: C, 51.89; H, 6.90; N, 8.37; Cl, 15.87%.

10-(3-Aminopropoxy)-4,5-dimethoxy-2,3,12,13-tetrahydro-1*H*-benzo[*c*]pyrido[2,3,4-*kl*]acridine Hydrochloride (5b)

Yield 41%; red solid: mp > 250°C (MeOH/Et₂O); IR (KBr) 1650, 3404 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.09 (qt, J = 6.9, 2H), 2.95–2.98 (m, 6H), 3.22 (t, J = 6.3, 2H), 3.46 (t, J = 5.8, 2H), 3.75 (s, 3H), 3.97 (s, 3H), 4.23 (t, J = 5.8, 2H), 6.78 (sl, 2H), 7.11 (s, 1H), 7.15 (d, J_o = 8.6, 1H), 7.47 (s, 1H), 8.15 (sl, 3H), 8.56 (d, J_o = 8.5, 1H); MS (EI) m/e 405 (M$^+$, 100), 390 (88), 333 (80), 290 (15); Anal. Calcd for $C_{24}H_{27}N_3O_3 \cdot 2HCl$ (3H₂O): C, 54.14; H, 6.63; N, 7.89; Cl, 13.31. Found: C, 54.39; H, 6.36; N, 7.85; Cl, 13.31%.

10-(3-Aminoethoxy)-4,5-dimethoxy-2,3,12,13-tetrahydro-1*H*-benzo[*c*]pyrido[2,3,4-*kl*]acridine Hydrochloride (5c)

Yield 35%; red solid: mp > 250°C (EtOH/Et₂O); IR (KBr) 1645, 3444 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.97–3.00 (m, 4H), 3.21–3.27 (m, 4H), 3.46 (t, J = 5.8, 2H), 3.75 (s, 3H), 3.97 (s, 3H), 4.32 (t, J = 5.9, 2H), 6.77 (sl, 2H), 7.14 (s, 1H), 7.16 (d, J_o = 8.6, 1H), 7.47 (s, 1H), 8.33 (sl, 3H), 8.56 (d, J_o = 8.6, 1H); MS (EI) m/e 391 (M$^+$, 88), 376 (68), 333 (100), 289 (16); Anal. Calcd for $C_{23}H_{25}N_3O_3 \cdot 2HCl$ (1H₂O): C, 57.26; H, 6.06; N, 8.71; Cl, 14.70. Found: C, 57.32; H, 6.12; N, 8.67; Cl, 14.61%.

RESULTS AND DISCUSSION

Chemistry

The pentacycles **4a** and **6a** (Chart 3) were prepared by a Friedländer reaction between the 5-amino-7,8-dimethoxy-2,3-dihydro-1*H*-quinolin-4-one **1** or **2** and the appropriate α-tetralone **3a** or **3d**. The starting materials **1** and **2** were obtained from 2,3-dimethoxybenzoic acid as previously described by the authors,[7] in seven and eight steps respectively. Acid hydrolysis of amide **4a** produced the secondary amine **5a** (Scheme 2). *O*-alkylation of **4d** with *N*-benzylcarbamoyl-ε-chloroalkylamines followed by catalytic

cleavage of Cbz group gave **4b**,**c** (Scheme 1) which provided **5b** or **5c** by acidic hydrolysis (Scheme 2). Benzopyridoacridines **6b**,**c** were obtained using the same strategy (Scheme 1): in this case *O*-alkylation involved *N*-Boc protected ε-chloroalkylamines.

Cell Culture and Growth Assay

Human prostate cancer PC-3 cells were used to assess the antiproliferative effect of the target benzopyridoacridines **4**–**6** and ASC was used as a positive control to compare with the tested compounds (Table I). After culture synchronisation, stimulation of quiescent cells was performed by epidermal growth factor and cell growth was stimulated by the colorimetric MTT test.

The results indicate that compounds **4a**, **5a** and **6b** possess excellent antiproliferative activity with IC$_{50}$ values in the nanomolar range. Among the new derivatives, compound **6b** emerges as the most active, with an IC$_{50}$ value of 5.1 nM comparable with that for ASC (2.1 nM). Compounds **4a** and **5a** were slightly less cytotoxic, with IC$_{50}$ < 20 nM. The obtained data enables us to formulate some preliminary structure–activity relationships concerning substitution in positions-3 and -10.

For the alkoxyamino side chain in position-10, it can be concluded that the optimal distance between the oxygen and the nitrogen atoms corresponds to three methylene groups, as indicated by the results obtained with **6b** *versus* **6c**. As cytotoxicity increased with the number of CH₂ units, this suggests that the extension of the alkoxyamino function, and therefore
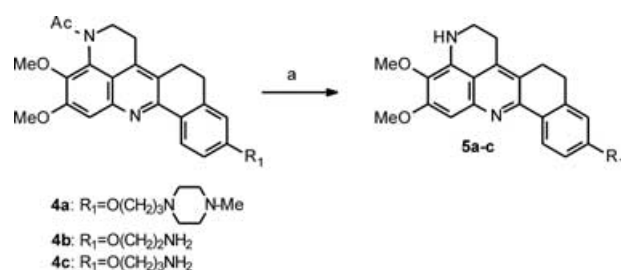
SCHEME 2 Conditions and reagents: (1) HCl 6N, reflux.

TABLE I *In vitro* antitumour activity against PC-3 cell line[a]

Compd	IC_{50} (nM)
4a[b]	11.2 ± 1.2
4b[b]	>10^3
4c[b]	>10^3
5a[b]	19.9 ± 1.3
5b[b]	>10^3
5c[b]	>10^3
6a[b]	>10^3
6b[b]	5.1 ± 0.5
6c[b]	>10^3
ASC[c]	2.1 ± 0.2

[a]3-day MTT assay; [b]Hydrochloride; [c]ASC is used as reference compound.

the relative lipophilicity of the C_{10} substituent, may contribute to the activity.

3-Acetylbenzopyridoacridine (**4a**) was more cytotoxic than the corresponding *N*-non substituted derivative (**5a**), which was more cytotoxic than the 3-piperazinylacetyl derivative (**6a**), suggesting that the length and/or relative lipophilicity of the N-3 substituent may influence the cytotoxicity.

Replacement of the terminal primary amine of the alkoxyamino side chain at C_{10} by the bulky *N*-methylpiperazinyl moiety increased cytotoxicity in **4** and **5** (i.e. comparison of **4a** with **4b,** and **5a** with **5b**) but decreased the cytotoxicity in **6** (**6a** *versus* **6b**). These findings suggest that the nature of N-3 substituents also influences activity.

CONCLUSION

This paper presents the first biological results on a readily accessible series of novel polycyclic acridines, in which the core heterocyclic framework is structurally related to bioactive natural products. On the basis of the results, it appears that some structural features of benzopyridoacridines such as relative lipophilicity, as well as size and nature of the terminal amino group of the cationic side chains at N_3 and C_{10} positions strongly influence cytotoxic activity. Compounds **4a**, **5a** and **6b** may target DNA and its enzymes. Their mode of action will be investigated in further studies.

References

[1] Molinski, T.F. (1993) *Chem. Rev.* **93**, 1825–1838.
[2] Bonnard, I., Bontemps, N., Lahmy, S., Banaigs, B., Combaut, G., Francisco, C., Colson, P., Houssier, C., Waring, M.J. and Bailly, C. (1995) *Anti-Cancer Drug Des.* **10**, 333–346.
[3] Lindsay, B.S., Barrows, L.R. and Copp, B.R. (1995) *Bioorg. Med. Chem. Lett.* **5**, 739–742.
[4] Suffness, M. and Cordell, G. (1985) In: Brossi, A., ed, *The Alkaloids* (Academic Press, New York), pp 178–198.
[5] Bhakkuni, D.S. and Jain, S. (1986) In: Brossi, A., ed, *The Alkaloids* (Academic Press, New York), pp 95–181.
[6] Pilch, D.S., Yu, C., Makhey, D., LaVoie, E.J., Srinivasan, A.R., Olson, W.K., Sauers, R.R., Breslauer, K.J., Geacintov, N.E. and Liu, L.F. (1997) *Biochemistry* **36**, 12542–12553.
[7] Chackal, S., Houssin, R. and Hénichart, J.-P. (2002) *J. Org. Chem.* **67**, 3502–3505.