Regiospecific Synthesis of Pyrido[3,4-*b*]- and Pyrido[4,3-*b*]carbazole-5,11-dione Derivatives. Evaluation of Their *In Vitro* Antifungal or Antiprotozoological Activities

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Hetero Diels–Alder reactions between 2- or 3-bromocarbazolequinones 1a or 1b and azadiene 5 afford regiospecifically pyrido[3,4-*b*]- and pyrido[4,3-*b*]carbazole-3,5,11-triones 6a and 6b. The regiochemistry of the cycloadditions is controlled by the position of the bromine atom at C-2 or C-3 of the bromoquinone. The corresponding *N*- and *O*-methyl derivatives 7 and 8 are prepared. Structural assignment of the regioisomers is made by ^{1}H-NMR nuclear Overhauser effect difference experiments performed on a diacetoxy derivative of pyrido[4,3-*b*]carbazole 9b. The *in vitro* antifungal and antiprotozoological activities of some prepared derivatives have been evaluated against *Candida albicans*, *Candida krusei*, *Cryptococcus neoformans* and *Trichomonas vaginalis*. None of the tested compounds have shown significant activity towards the yeasts or protozoa.

Key words bromocarbazolequinone; 2-azadiene; Diels–Alder; pyridocarbazolequinone; antifungal activity; antiprotozoological activity

Naturally occurring carbazole alkaloids such as murrayaquinones A—E (I),[1] pyrayaquinones A and B (II)[2] and clausenaquinone A (III)[3] possess a carbazolequinone skeleton substituted at C-3 by a methyl or a methoxyl group. Among these, murrayaquinone A have been reported to develop a cardiotonic activity on guinea pig papillary muscle,[4] while clausenaquinone A has shown inhibitory activity of rabbit platelet aggregation as well as cytotoxycity towards tumor cells.[3] Furthermore, several synthetic carbazolequinones have been described for their antibacterial and antifungal properties.[5] On the other hand, some plant alkaloids such as ellipticine IVa and its 9-methoxy derivative IVb have in their structure a pyridine ring fused to carbazole (Chart 1). Since their isolation[6] and the discovery of their anticancer activity,[7] synthetic approaches to pyridocarbazolequinone systems directed to their conversion into ellipticine or isoellipticine have been achieved using annelated methods.[8] In order to assess the antifungal or antiparasitical properties of the pyridocarbazolequinone structure, we planned to synthesize some new compounds V which have a nitrogen atom in a to d positions with substituents on the pyridine ring (Chart 2). Recently, we reported a direct access to pyrido[2,3-*b*]- and pyrido[3,2-*b*]carbazole-5,11-diones, **3** and **4** respectively, through [4+2] cycloaddition reactions between carbazolequinones **1** and α,β-unsaturared *N*,*N*-dimethylhydrazones **2**.[9] In continuation of our program, we describe in this paper the synthesis of pyrido[3,4-*b*]- and pyrido[4,3-*b*]carbazolequinone derivatives **6a** and **6b** *via* a regiospecific hetero Diels–Alder reaction using azadiene **5** and carbazolequinones **1a** or **1b** (Chart 3). To our knowledge, the cycloaddition between a 2-azadiene and heterocyclic quinones has not yet been reported.

Synthesis Our first attempts started with quinone **1c**. The kinetics of its cycloaddition towards azadiene **5** were slow. Indeed, less than 5% of the regioisomeric cycloadducts **6** were obtained after reflux for 48 h in chloroform or for 24 h in toluene. Moreover, a large amount of degradation products was formed. Using 2- or 3-bromocarbazolequinones **1a** or **1b** directly and regiospecifically afforded the corresponding pyridocarbazolequinones **6a** or **6b** in an excellent yield (96 and 95%, respectively). These two regioisomers are differentiated by their ^{1}H-NMR spectral data. More particularly, the

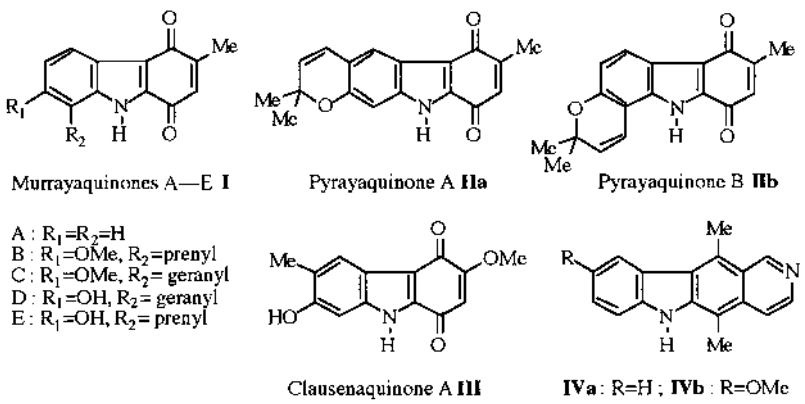

Chart 1

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larger $\Delta\delta$=0.11 ppm is observed for the N–H signals (δ= 12.40 ppm and 12.29 ppm for **6a** and **6b**, respectively).

Then, methylation of the lactam function was performed on the nitrogen atom by treatment of compounds **6** with dimethyl sulfate in the presence of anhydrous potassium carbonate in *N*,*N*-dimethylformamide (DMF), while *O*-methyl derivatives were selectively formed by the use of silver oxide and methyl iodide in tetrahydrofuran (THF) (Chart 4).

To assign the structure for the regioisomers, pyridocarbazolequinone **8b** was converted to the diacetoxyl derivative **9b** following a known procedure[10] (Chart 5). Then, a ^{1}H-NMR nuclear Overhauser effect difference experiment performed on **9b** proved its regiochemistry (Chart 6). Thus, irradiation of the methyl signal at 2.68 ppm (11-OAc) gave two responses: one at 9.1 ppm (H-1) and the other one at 7.97 ppm (H-10). Then, irradiation of the signal at 2.56 ppm (5-OAc) afforded three responses: one on H-4 (δ=6.84 ppm), one on the CH_2 of the ethyl group (δ=4.41 ppm) and the third one on the CH_3 of the ethyl group (δ=1.47 ppm). These results were confirmed by the irradiation of H-4 (δ=6.84 ppm) which gave, on one hand, a response on the methoxyl substituent at 4.06 ppm and, on the other hand, another response on the methyl signal of 5-OAc (δ=2.56 ppm). Moreover, irradiation of H-10 at 7.97 ppm gave responses on H-9 (δ=7.32

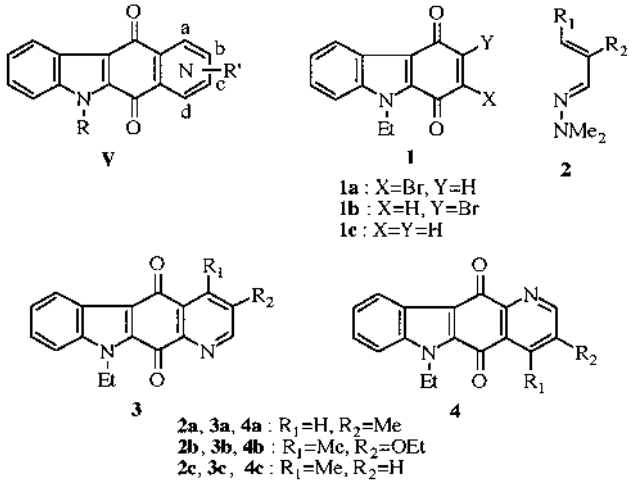

Chart 2

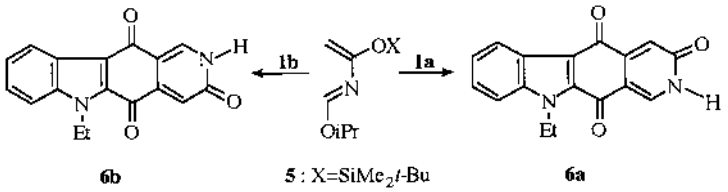

Chart 3

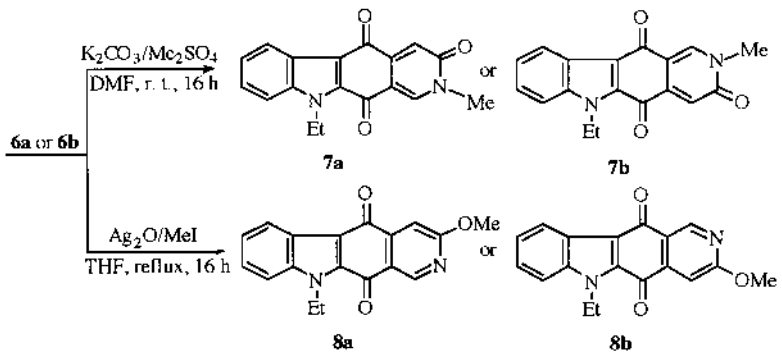

Chart 4

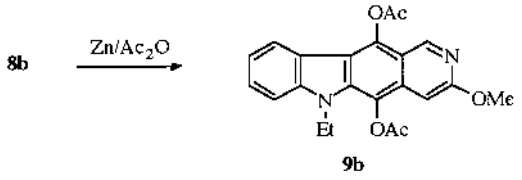

Chart 5

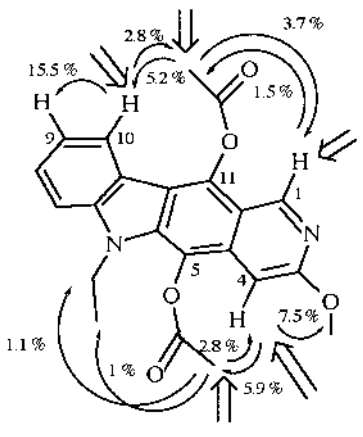

Chart 6

ppm) and CH_3 of 11-OAc (δ=2.68 ppm), while irradiation of H-1 at δ=9.1 ppm provided a response on CH_3 of 11-OAc.

In order to explain the orientation of the cycloadditions, we calculated the HOMO and LUMO orbital coefficients for azadiene **5** and quinones **1a** and **1b** by the semi-empirical method PM3.[11] Thus, for azadiene **5**, the larger HOMO coefficient is located at C-4 (the values are 0.236 for C-1 and 0.691 for C-4) while for quinones **1a** and **1b** those of the LUMO are situated at C-3 (**1a**: C-2=0.3277, C-3=0.3757 and **1b**: C-2=0.3393, C-3=0.3645). The regiochemistry observed in these cycloadditions cannot be explained by molecular orbital frontier considerations, but it agrees with the blocking effect of the bromine atom.[9] Indeed, the nucleophilic end (C-4) of azadiene **5** attacks the unbrominated carbon of the quinones.

Pharmacology *In Vitro* Antifungal Assays: The antifungal assays were performed *in vitro* against three strains of yeasts: *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans*. Among the prepared compounds, only **3a**, **4a** and **4c** were soluble in dimethyl sulfoxide (DMSO) at the concentration of 0.2%. None of these derivatives have exhibited an interesting antifungal activity against these yeasts (inhibitory minimum concentration (IMC>200 μg/ml).

In Vitro Antiprotozoological Assays: The antiprotozoological assays were performed *in vitro* against strains of *Trichomonas vaginalis*. Dimetridazole, used as a reference, was active at a minimum lethal concentration (MLC) of 10 μg/ml, while the soluble compounds tested, **3a**, **3c** and **8b**, were found to be inactive.

Experimental

Melting points were measured on a Büchi apparatus (capillary tube). The infrared (IR) spectra were obtained on a Perkin–Elmer 1310 spectrophotometer. The ^{1}H-NMR spectra were recorded at 300 MHz on a Bruker AM 300 apparatus. Chemical shifts are reported in ppm (δ) using tetramethylsilane (TMS) as an internal reference. Coupling constant (J) values are given in Hz. Elemental analyses were done at the Centre de Microanalyse du CNRS at Solaize, France. Coefficients of the molecular frontier orbitals were calculated from MOPAC of the SYBYL program on an IBM Risk 6000 workstation.

2-Bromocarbazolequinone **1a**,[9] 3-bromocarbazolequinone **1b**[9] and azadiene **5**,[12] were prepared according to the respective cited procedures.

Cycloadditions of Azadiene 5 to Bromoquinones 1a and 1b A solution of azadiene **5** (1.34 g, 5.51 mmol) and bromoquinone **1a** or **1b** (0.67 g, 2.2 mmol) in chloroform (16 ml) was stirred and heated to reflux under argon for 12 h. Then, chloroform was evaporated and the residue immediatly dissolved in acetone (15 ml) and heated to reflux for 2 h. An orange precipitate was formed. It was recovered and washed with acetone.

10-Ethyl-2*H*-pyrido[3,4-*b*]carbazole-3,5,11(10*H*)-trione (6a) Compound **6a** was obtained in 96% yield, mp>300 °C. IR (KBr): 1675—1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 80 °C) δ: 12.40 (1H, br s, NH), 8.32 (1H, d, J=7.8 Hz, H-6), 8.24 (1H, s, H-1), 7.83 (1H, d, J=8.3 Hz, H-9), 7.56 (1H, m, H-7 or H-8), 7.41 (1H, m, H-7 or H-8), 6.88 (1H, s, H-4), 4.82 (2H, q, J=7.0 Hz, C$\underline{H}_2$CH$_3$), 1.42 (3H, t, J=7.0 Hz, CH$_2$C$\underline{H}_3$). *Anal*. Calcd for $C_{17}H_{12}N_2O_3\cdot 0.8\,H_2O$: C, 66.57; H, 4.47; N, 9.13. Found: C, 66.55; H, 4.15; N, 9.09.

6-Ethyl-2*H*-pyrido[4,3-*b*]carbazole-3,5,11(6*H*)-trione (6b) Compound **6b** was obtained in 95% yield, mp>300 °C. IR (KBr): 1670—1640 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 80 °C) δ: 12.29 (1H, br s, NH), 8.35 (1H, d, J=8.1 Hz, H-10), 8.15 (1H, s, H-1), 7.80 (1H, d, J=8.1 Hz, H-7), 7.56 (1H, m, H-8 or H-9), 7.42 (1H, m, H-8 or H-9), 6.92 (1H, s, H-4), 4.75 (2H, q, J=7.0 Hz, C$\underline{H}_2$CH$_3$), 1.41 (3H, t, J=7.0 Hz, CH$_2$C$\underline{H}_3$). *Anal*. Calcd for $C_{17}H_{12}N_2O_3\cdot 0.7\,H_2O$: C, 66.96; H, 4.43; N, 9.18. Found: C, 66.77; H, 4.16; N, 9.10.

10-Ethyl-2-methyl-2*H*-pyrido[3,4-*b*]carbazole-3,5,11(10*H*)-trione (7a) To a solution of **6a** (0.08 g, 0.27 mmol) in DMF (100 ml) were added K_2CO_3 (0.112 g, 0.81 mmol) and dimethyl sulfate (0.17 g, 4.05 mmol). The resulting mixture was stirred overnight at room temperature. Then, the solution was diluted with water (30 ml) and extracted with CH_2Cl_2 (3×20 ml). The organic layer was washed with water and dried over $MgSO_4$. The solvent was evaporated and the residue recrystallized from DMF. Compound **7a** was obtained as a red powder in 66% yield, mp>300 °C. IR (KBr): 1675, 1640, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 60 °C) δ: 8.72 (1H, s, H-1), 8.29 (1H, d, J=8.2 Hz, H-6), 7.87 (1H, d, J=8.1 Hz, H-9), 7.57 (1H, m, H-7 or H-8), 7.46 (1H, m, H-7 or H-8), 6.90 (1H, s, H-4), 4.82 (2H, q, J=6.9 Hz, C$\underline{H}_2$CH$_3$), 3,63 (3H, s, N–CH$_3$), 1.40 (3H, t, J=6.9 Hz, CH$_2$C$\underline{H}_3$). *Anal*. Calcd for $C_{18}H_{14}N_2O_3\cdot 0.1\,H_2O$: C, 70.16; H, 4.64; N, 9.09. Found: C, 69.84; H, 4.60; N, 9.13.

6-Ethyl-2-methyl-2*H*-pyrido[4,3-*b*]carbazole-3,5,11(6*H*)-trione (7b) To a solution of **6b** (0.065 g, 0.22 mmol) in DMF (80 ml) were added K_2CO_3 (0.092 g, 0.67 mmol) and dimethyl sulfate (0.140 g, 1.11 mmol). The resulting mixture was stirred overnight at room temperature and treated as above. After recrystallization from DMF, compound **7b** was obtained as a red powder in 67% yield, mp>300 °C. IR (KBr): 1670, 1655, 1625 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 60 °C) δ: 8.58 (1H, s, H-1), 8.36 (1H, d, J=8.5 Hz, H-10), 7.81 (1H, d, J=7.9 Hz, H-7), 7.57 (1H, m, H-8 or H-9), 7.43 (1H, m, H-8 or H-9), 6.94 (1H, s, H-4), 4.76 (2H, q, J=7.1 Hz, C$\underline{H}_2$CH$_3$), 3.63 (3H, s, N–CH$_3$), 1.40 (3H, t, J=7.1 Hz, CH$_2$C$\underline{H}_3$). *Anal*. Calcd for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.60; N, 9.14. Found: C, 70.29; H, 4.7 ; N, 9.29.

10-Ethyl-3-methoxypyrido[3,4-*b*]carbazole-5,11(10*H*)-dione (8a) A solution of **6a** (0.368 g, 1.26 mmol) in THF (600 ml) was stirred and heated to reflux. Then, Ag_2O (1 g, 4.31 mmol) and methyl iodide (3.6 g, 25.3 mmol) were added and the reflux was maintained for 16 h. After filtration through Celite, the solvent was removed under vacuum and the residue purified by column chromatography using $CHCl_3$/EtOAc (98 : 2) as the eluent. The resulting yellow solid was recrystallized from acetone. It was obtained in 72% yield, mp 205 °C. IR (KBr): 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 8.95 (1H, s, H-1), 8.40 (1H, d, J=7.9 Hz, H-6), 7.48—7.33 (4H, m, H arom.), 4.77 (2H, q, J=7.1 Hz, C$\underline{H}_2$CH$_3$), 4.05 (3H, s, OCH$_3$), 1.50 (3H, t, J=7.1 Hz, CH$_2$C$\underline{H}_3$). *Anal*. Calcd for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.60; N, 9.14. Found: C, 70.52; H, 4.67; N, 9.07.

6-Ethyl-3-methoxypyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (8b) Compound **8b** was prepared following the procedure used for **8a**, from 3-oxopyridocarbazolequinone **6b** (0.1 g, 0.326 mmol), methyl iodide (0.5 g, 3.5 mmol) and Ag_2O (1.5 g, 6.47 mmol) in THF (200 ml). Compound **8b** was obtained in 55% yield, mp 247 °C. IR (KBr): 1660, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 9.01 (1H, s, H-1), 8.48 (1H, d, J=8 Hz, H-10), 7.50 to 7.34 (4H, m, H arom.), 4.76 (2H, q, J=7.2 Hz, C$\underline{H}_2$CH$_3$), 4.08 (1H, s, OCH$_3$), 1.49 (3H, t, J=7.2 Hz, CH$_2$C$\underline{H}_3$). *Anal*. Calcd for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.60; N, 9.14. Found: C, 70.71; H, 4.65; N, 8.95.

5,11-Diacetoxy-6-ethyl-3-methoxy-6*H*-pyrido[4,3-*b*]carbazole (9b) A suspension of **8b** (0.05 g, 0.163 mmol), zinc powder (0.20 g, 3 mmol) and sodium acetate (0.0895 g, 1.03 mmol) in 25 ml of acetic anhydride was stirred at room temperature for 15 min. Then, CH_2Cl_2 (100 ml) was added and the mixture was filtered. The solvent was removed under a vacuum and the residue recrystallized from EtOAc. Compound **9b** was obtained as a fluorescent yellow powder in 42% yield, mp 221 °C. IR (KBr): 1755 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 9.10 (1H, s, H-1), 7.97 (1H, d, J=7.7 Hz, H-10), 7.54 (1H, m, H-8), 7.35—7.27 (2H, m, H-7 and H-9), 6.84 (1H, s, H-4), 4.41 (2H, q, J=7.3 Hz, C$\underline{H}_2$CH$_3$), 4.06 (3H, s, OCH$_3$), 2.68 (3H, s, OCOCH$_3$-11), 2.56 (3H, s, OCOCH$_3$-5), 1.47 (3H, t, J=7.3 Hz, CH$_2$C$\underline{H}_3$). *Anal*. Calcd for $C_{22}H_{20}N_2O_5$: C, 67.33; H, 5.14; N, 7.13. Found: C, 67.32; H, 5.18; N, 6.92.

***In Vitro* Antifungal Assays** The following species from the Department of Parasitology, Medical Mycology and Animal Organization of the Faculty of Pharmacy, University Claude Bernard of Lyon, were used for the *in vitro* screening tests: *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans*.

All cultures were performed in plates for cell research work, Nunclon TM Δ (NUNC Company) with RPMI 1640 MOPS medium, according to the Datry *et al*. method.[13] A sterile aqueous solution of fluconazole used as a control, and compounds dissolved in DMSO (final concentration of DMSO=0.2%) were tested at different concentrations (from 200 to 3 μg/ml).

Yeasts were spot inoculated in the medium to a concentration of approximately 1.5×10^3 per ml. Plates were incubated at 35 °C for 2 d for the two species of *Candida* and 3 d for *Cryptococcus neoformans*.

Classically, for each culture, IMC was evaluated by the diminution of turbidimetry.

***In Vitro* Antiprotozoological Assays** The *in vitro* antiparasitical assays were performed against twelve axenic strains of *Trichomonas vaginalis* isolated following the procedure of Andrew *et al*.[14] Diamond modified medium (6 g trypticase, 1.5 g maltose, 0.06 g ascorbic acid, 0.3 g yeast extract, 0.3 g cysteine HCl, 225 ml distilled water, 10% bovine serum, 1000 U/ml peni-

cillin G, 1000 mg/ml streptomycin sulfate and 2 U/ml nystatin) was used for the maintenance and sensitivity assay of *Trichomonas vaginalis* isolates.

Dimetridazole (DMZ), supplied by Farmiga SpA, Pisa, was used as the control. A 30% sterile stock solution of this drug (DMZ 30) was prepared in DMSO according to the instructions suggested by Juliano *et al.*[15]

Cell numbers were determined using a Neubauer chamber.

A 48-h-old culture of *Trichomonas vaginalis* was collected by centrifugation and resuspended in pre-warmed medium to obtain about 2×10^6 cells/ml. Antimicrobial agents were diluted in tests tubes and then added to the culture media as follows: 4 ml of *T. vaginalis* broth was mixed with 0.5 ml of the culture and 0.5 ml of the antibiotic solution. Control tubes similarly prepared contained the microorganism without the antimicrobial agent. The samples were incubated at 37 °C under aerobic conditions to maximize dimetridazole resistance. The growth of the microorganisms was checked after 48 h by a phase-contrast microscope for protozoan motility. MLC was defined as the minimum concentration of the drug at which no motile trichomonas were observed. The tested compounds were dissolved in DMSO (final concentration of DMSO=0.2%).

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