Synthesis of Carbazolequinone Derivatives as Inhibitors of *Toxoplasma gondii* Purine Nucleoside Phosphorylase

Zouhair Bouaziz,[a] Arnaud Ghérardi,[b] François Régnier,[a] Marie-Elizabeth Sarciron,[b] Xavier Bertheau,[a] Bernard Fenet,[c] Nadia Walchshofer,[a] and Houda Fillion*[a]

Keywords: Alkaloids / Natural products / Cycloadditions / Nitrogen heterocycles / PNP inhibitors

9-Ethyl-6-hydroxycarbazolequinone (**9**) was synthesized and submitted to a hetero Diels–Alder reaction towards azadienes **10a** or **10b** to afford the hydroxypyridocarbazole-5,11-diones **11** or **12a**,**b**. A Bracher cyclization applied to compound **12b** led to the 9-hydroxyquinoneimine **15** admixed with its 9-methyl ether **16**. These compounds as well as other carbazolequinone derivatives were evaluated towards a purine nucleoside phosphorylase isolated from two strains of *Toxoplasma gondii* (a virulent strain RH and a cystic strain ME 49). The carbazolequinones **1a**, **1b**, **1d**, **9** and pyridocarbazolequinones **2a**, **4** and **5** have shown inhibitory activities similar or better than those observed with the reference compound 8-aminoguanosine.

Introduction

The carbazolequinone skeleton is present in various naturally occurring alkaloids. Moreover, it is an interesting precursor[1a] for many other heterocyclic compounds such as pyridocarbazole derivatives of pharmacological interest. Recently, we described a direct access to the pyridocarbazole-5,11-diones **2**, **3**, **4a** and **4b** through a hetero Diels–Alder reaction between carbazolequinones **1** and the appropriate azadienes.[1b,1c] The lactam compounds **4** were then converted into their corresponding *O*-methyl derivatives **5** (Scheme 1).

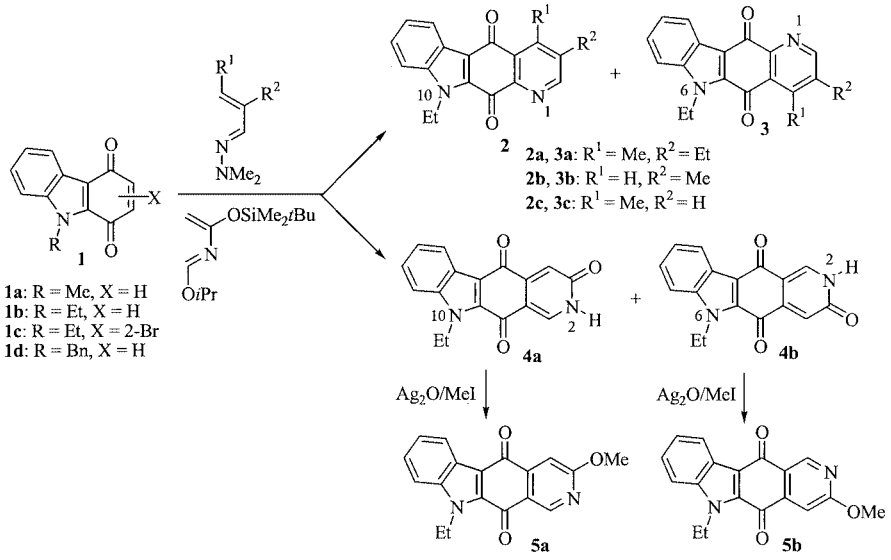
We are also engaged in a search for active drugs against *Toxoplasma gondii*.[2] In a continuation of this work, we were interested in introducing a hydroxy group at C-7 of pyrido[2,3-*b*]- or at C-9 of pyrido[3,2-*b*]carbazole-5,11-diones. The presence of such a substituent should improve the biological properties. For this purpose, we planned to prepare a 6-hydroxycarbazolequinone derivative and to use this new dienophile in [4+2] cycloadditions towards 1-azadienes. Then, we aimed to evaluate the inhibitory activity of the prepared carbazolequinones and pyridocarbazole derivatives against *Toxoplasma gondii* purine nucleoside phosphorylase (PNP, E.C. 2.4.2.1.). The latter, identified for the first time in our group,[3] is an enzyme of the purine salvage pathway, which is the most striking metabolic difference between protozoa and humans.[4] The inhibition of PNP could, therefore, represent a novel chemotherapy strategy.

[a] Laboratoire de Chimie Organique EA 635, Université Claude Bernard, Faculté de Pharmacie,
8 Avenue Rockefeller, 69373 Lyon Cedex 08, France
Fax: (internat.) + 33-4/78777158
E-mail: fillion@cismsun.univ-lyon1.fr
[b] Laboratoire de Parasitologie EA 1887, Université Claude Bernard, Faculté de Pharmacie,
8 Avenue Rockefeller, 69373 Lyon Cedex 08, France
[c] Centre de RMN, Université Claude Bernard,
69622 Villeurbanne Cedex, France

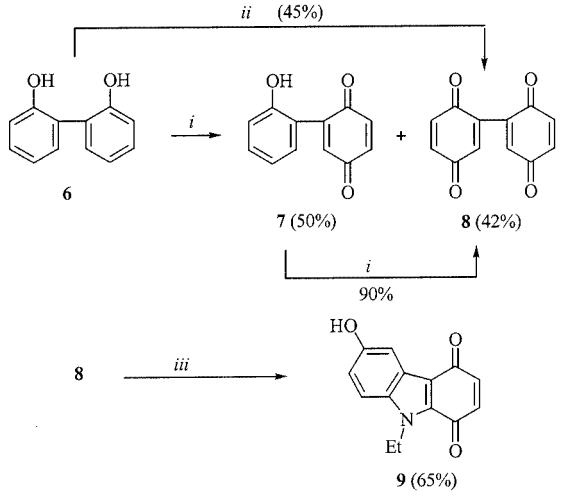
Synthesis

6-Hydroxycarbazolequinone **9** was prepared in two steps from commercially available 2,2′-dihydroxybiphenyl (**6**) through its oxidation to 2,2′,5,5′-biphenyldiquinone (**8**)[5] followed by the reaction of **8** with ethylamine (Scheme 2). To oxidize **6**, we employed two methods. In method A, oxidation of **6**, performed with Frémy's salt, afforded a separable mixture of 1-(2′-hydroxyphenyl)-2,5-benzoquinone (**7**)[6] and diquinone **8**. Then, further treatment of **7** with Frémy's salt gave **8** in 90% yield. A more direct procedure (method B) provided the single product **8** in 45% yield, through the oxidation of **6** with lead dioxide in the presence of perchloric acid. To obtain carbazolequinone **9**, and to avoid a further nucleophilic addition of ethylamine to **9**, a stoichiometric amount of this amine was treated with diquinone **8**.

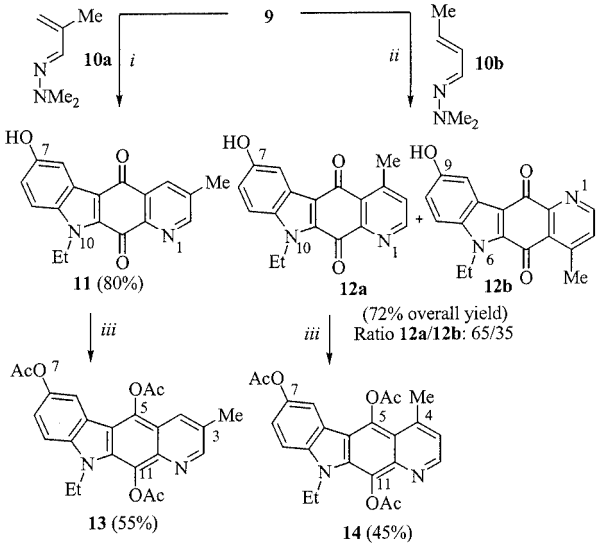
The [4+2] cycloaddition between azadiene **10a**[7,8] and carbazolequinone **9** (EtOH, reflux, 2 h) led to the hydroquinone tautomer[7b] of the initial 1:1 cycloadduct, which, after loss of dimethylamine, was oxidized by air to quinone **11**. In contrast, the reaction of azadiene **10b**[9] with **9** (toluene, reflux, 1 h) afforded a mixture of the regioisomeric dihydro adducts,[10] which were aromatized to **12a** and **12b** by addition of basic alumina in situ (Scheme 3). The



Scheme 1. Aza Diels–Alder cycloadditions



Scheme 2. Synthesis of carbazole quinone **9**: (*i*, Method A): Frémy's salt, acetone/water, room temp.; (*ii*, Method B): $PbO_2/HClO_4$, acetonitrile; (*iii*): $EtNH_2$, $CHCl_3$, 0 °C



Scheme 3. Cycloadditions of carbazolequinone **9**: (*i*): EtOH, reflux, 2 h; (*ii*): 1) toluene, reflux, 1 h, 2) Al_2O_3, reflux, 12 h; (*iii*): Ac_2O, Zn/AcONa

aromatized adducts were then easily separated by column chromatography on silica gel (ratio **12a/12b** = 65:35) and identified from their spectroscopic data.

To assign the structure of compound **11** we performed a reductive acetylation with zinc powder in the presence of acetic anhydride and sodium acetate. Then, a ^1H NMR nuclear Overhauser effect difference (NOE DIFF) experiment carried out on the triacetoxy derivative **13** allowed us to confirm the regiochemistry of the Diels–Alder reaction (Figure 1). Applying the same experimental conditions reported above, the major product **12a** gave the triacetoxy derivative **14**. A ^1H NMR transient NOE experiment,[11] performed on **14**, allowed us to assign its structure.

In order to synthesize indole analogues of sampangine, a natural naphthonaphthyridine-7-one, we applied the Bracher cyclization[12] to pyridocarbazolequinones **12**. Thus, compound **12b** was treated with dimethylformamide

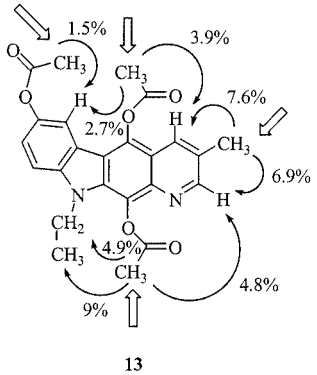
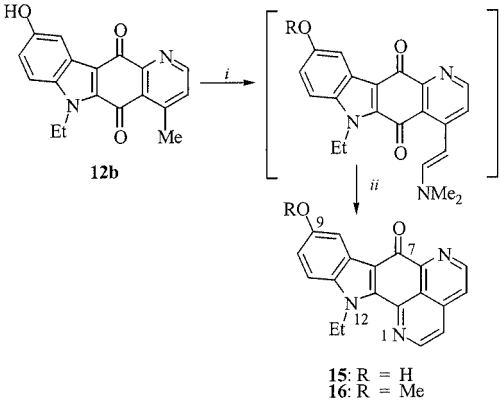


Figure 1. ^1H NMR NOE DIFF experiments performed on the triacetylated compound **13**

dimethylacetal (DMF DMA) in DMF, followed by addition of ammonium chloride and acetic acid, to afford the corresponding quinoneimine **15**. The latter was obtained admixed with its methyl ether **16** (Scheme 4). The *O*-methyl derivative was formed in the reaction mixture through a transetherification with DMF DMA.[13] Starting with the regioisomer **12a**, our attempts to obtain an analogous cyclized product failed. This failure can be explained by the donor effect of the indole nitrogen atom, which, in **12a**, increases the electron density at the carbon atom (C-5) close to the pyridine methyl substituent.



Scheme 4. Bracher's cyclisation of pyridocarbazolequinone **12b**: (*i*): DMF DMA, DMF, N_2, 110 °C, 3 h; (*ii*): NH_4Cl, AcOH, DMF, N_2, 110 °C, 1 h

Biological Results

The PNP inhibition activity of some of the above compounds was studied on two strains of *Toxoplasma gondii* (a virulent strain RH and a cystic strain ME 49) with 8-aminoguanosine, a *Plasmodium falciparum* and human PNP inhibitor,[14] formycine A, an *E. coli* PNP inhibitor,[15] and allopurinol riboside, a substrate of human PNP,[16] as the reference compounds. The results are shown in Table 1. We found that 8-aminoguanosine inhibits the PNP of both *T. gondii* strains with a K_i value of 0.2 mM. Among the tricyclic quinones tested, those unsubstituted at the C-2−C-3 double bond (**1a**, **1b**, **1d** and **9**) show a similar or lower K_i value to that obtained with 8-aminoguanosine, our best reference compound. Among them, the *N*-ethyl derivative **1b** is the most active compound. Concerning the tetracyclic quinones, the presence of a hydroxy group in compounds **11**, **12a**, and **12b** does not improve the inhibitory activities relative to those of the non-hydroxylated derivatives **2a** and **3b**. In some cases, the position of the nitrogen atom in the pyridine ring has an influence on the K_i values, the best activities being observed with the 1,10-regioisomers **2a** and **12a** rather than with the 1,6-regioisomers **3b** and **12b**. Finally, quinones **5a** and **5b** are more active than the corresponding lactam derivatives **4a** and **4b**.

Table 1. PNP inhibitory activity of synthesized compounds; the values are the means ± S.D. of triplicate experiments

Compounds	K_i(RH) [mM]	K_i(ME 49) [mM]
1a	0.20	0.15
1b	0.07	0.14
1c	0.41	0.54
1d	0.11	0.23
2a	0.28	0.28
3b	0.97	0.60
4a	0.24	0.24
4b	0.25	0.24
5a	0.12	0.18
5b	0.10	0.30
9	0.30	0.17
11	0.50	0.26
12a	0.49	0.63
12b	1.38	1.02
15	0.86	0.90
16	0.84	0.88
8-aminoguanosine	0.20	0.20
Formycine A	1.68	1.70
Allopurinol ribose	2.00	1.80

Conclusion

In conclusion, we have prepared 6-hydroxycarbazolequinone **9** by oxidation of 2,2′-dihydroxybiphenyl with Frémy's salt or with lead dioxide in perchloric acid. Then, cycloaddition of **9** with azadienes **10a** or **10b** provided the pyridocarbazole-5,11-diones **11** or **12a**,**b**, respectively. An indole analogue of sampangine **15** and its *O*-methyl derivative **16** were prepared by a Bracher cyclization of **12b**. The prepared carbazolequinone derivatives were evaluated towards a purine nucleoside phosphorylase isolated from two strains of *Toxoplasma gondii*. We found that both the tricyclic quinones **1a**, **1b**, **1d** and **9** and the tetracyclic ones **2a**, **4** and **5** inhibit the enzyme with K_i values similar or lower than that obtained with our best reference compound.

Experimental Section

General: Melting points were measured with a Büchi apparatus (capillary tube). The IR spectra were recorded with a Perkin−Elmer 1310 spectrophotometer. The ^1H NMR spectra were recorded at 300 MHz with a Bruker AM 300 spectrometer. 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 performed at the Centre de Microanalyse du CNRS at Solaize, France.

9-Benzyl-1,4-dihydrocarbazole-1,4(9*H*)-dione (1d): A mixture of *N*-benzylanilinobenzoquinone (0.100 g, 0.35 mmol) and $Pd(OAc)_2$ (0.155 g, 0.7 mmol) in acetonitrile (5 mL) was heated under nitrogen to reflux for 2 h. After cooling, the reaction mixture was filtered and the solvent removed. The residue was purified by column chromatography on silica gel with a mixture of petroleum ether and Et_2O (80:20) as the eluent. Quinone **1d** was obtained as a red powder in 66% yield (0.065 g). M.p. 168−171 °C. IR (KBr): $\tilde{\nu}$ = 1655, 1640 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 5.85 (s, 2 H, $CH_2C_6H_5$), 6.63 (AB system, 2 H, *J* = 10.2 Hz, 2-H and 3-H), 7.45−7.13 (m, 8 H, H aromat.), 8.30 (d, *J* = 7.0 Hz, 1 H, 5-H) ppm. $C_{19}H_{13}NO_2$ (287.3): calcd. C 79.42, H 4.56, N 4.87; found C 79.01, H 4.59, N 5.03.

1-(2′-Hydroxyphenyl)-2,5-benzoquinone (7) and 2,2′,5,5′-Biphenyldiquinone (8).[5] **Method A:** A mixture of Frémy's salt (8 g, 30 mmol) and KH_2PO_4 (0.610 g, 4.5 mmol) in distilled water (250 mL) was added dropwise to a solution of 2,2′-dihydroxybiphenyl (0.930 g, 5 mmol) in acetone (75 mL). The reaction mixture was stirred at room temperature for 3 h and then extracted with CH_2Cl_2 (4 × 100 mL). After the usual workup, the residue, which contained 1-(2′-hydroxyphenyl)-2,5-benzoquinone (**7**) admixed with diquinone **8**, was column-chromatographed on silica gel with a mixture of CH_2Cl_2 and EtOAc (98:2) as the eluent. Compound **7** was obtained in 50% yield (0.500 g) while diquinone **8**, which eluted first, was isolated in 42% yield (0.450 g). Further oxidation of **7** with Frémy's salt gave **8** in 90% yield. **Method B:** A solution of 2,2′-dihydroxybiphenyl (0.744 g, 4 mmol) in acetonitrile (15 mL) was added dropwise to a cooled (in an ice-bath with salt) and stirred mixture of PbO_2 (2.4 g, 10 mmol), 70% $HClO_4$ (5 mL) and acetonitrile (15 mL). Stirring was maintained for 30 min. After filtration of the reaction mixture, extraction with CH_2Cl_2 and the usual workup, a column chromatography on silica gel with a mixture of CH_2Cl_2 and EtOAc (95:5) as the eluent gave **8** in 45% yield (0.385 g).

7: Orange powder, m.p. 170 °C (ref.:[6] 185 °C). IR (KBr): $\tilde{\nu}$ = 3280, 1660, 1640 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 7.0−6.83 (m, 5 H), 7.14 (d, *J* = 7.5 Hz, 1 H, 3′-H or 6′-H), 7.26 (dd, *J* = 7.5 Hz, 1 H, 4′-H or 5′-H), 9.73 (s, 1 H, OH) ppm. HRMS: calcd. for $C_{12}H_8O_3$ 200.04734; found 200.04731.

8: Yellow powder, m.p. 180 °C. IR (KBr): $\tilde{\nu}$ = 1650 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 6.85 (AB system 4 H, *J* = 12.5 Hz, 3-H, 4-H, 3′-H and 4′-H), 6.87 (s, 2 H, 6-H and 6′-H) ppm.

9-Ethyl-6-hydroxy-1,4-dihydrocarbazole-1,4(9*H*)-dione (9): A 2 M solution of ethylamine (1.4 mL, 2.8 mmol) in methanol was diluted with $CHCl_3$ (5 mL) and then added dropwise to a stirred solution of diquinone **8** (0.6 g, 2.8 mmol) in $CHCl_3$ (100 mL) at 0 °C. Stirring was maintained for 1 h. The organic layer was then washed with an aqueous solution of 5% HCl (3 × 50 mL) and dried with Na_2SO_4. The residue was purified by column chromatography on silica gel with a mixture of CH_2Cl_2 and EtOAc (90:10) as the eluent. Carbazolequinone **9** was obtained as a red powder in 65% yield (0.439 g). M.p. 225 °C. IR (KBr): $\tilde{\nu}$ = 3280, 1660, 1625 cm^{-1}. ^1H NMR ([D$_6$]DMSO): δ = 1.31 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3), 4.56 (q, *J* = 7.0 Hz, 2 H, CH_2CH_3), 6.65 (AB system, 2 H, *J* = 10.5 Hz, 2-H and 3-H), 6.95 (dd, *J* = 8.8, 2.2 Hz, 1 H, 7-H), 7.43 (d, *J* = 2.2 Hz, 1 H, 5-H), 7.60 (d, *J* = 8.8 Hz, 1 H, 8-H), 9.63 (s, 1 H, OH) ppm. $C_{14}H_{11}NO_3 \cdot 0.3H_2O$ (246.7): calcd. C 68.17, H 4.74, N 5.67; found C 68.17, H 4.71, N 5.03.

10-Ethyl-7-hydroxy-3-methyl-10*H*-pyrido[2,3-*b*]carbazole-5,11-dione (11): A solution of azadiene **10a** (0.160 g, 1.42 mmol) in ethanol (5 mL) was added dropwise to a stirred solution of carbazolequinone **9** (0.230 g, 0.954 mmol) in ethanol (20 mL). The reaction mixture was then heated to reflux for 2 h. After removing the solvent, the residue was purified by column chromatography on silica gel with CH_2Cl_2/MeOH (95:5) and then cyclohexane/EtOAc (1:2) as eluents. Compound **11** was obtained in 80% yield (0.234 g). M.p. 290 °C. IR (KBr): $\tilde{\nu}$ = 3340, 1660, 1640 cm^{-1}. ^1H NMR ([D$_6$]DMSO): δ = 1.37 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3), 2.46 (s, 3 H, 3-CH_3), 4.68 (q, *J* = 7.0 Hz, 2 H, CH_2CH_3), 7.06 (dd, *J* = 8.8, 2.2 Hz, 1 H, 8-H), 7.59 (d, *J* = 2.2 Hz, 1 H, 6-H), 7.63 (d, *J* = 8.8 Hz, 1 H, 9-H), 8.17 (d, *J* = 2.2 Hz, 1 H, 4-H), 8.75 (d, *J* = 2.2 Hz, 1 H, 2-H), 9.83 (s, 1 H, OH) ppm. $C_{18}H_{14}N_2O_3 \cdot 0.6H_2O$ (317.1): calcd. C 68.17, H 4.83, N 8.83; found C 68.23, H 4.85, N 8.50.

10-Ethyl-7-hydroxy-4-methyl-10*H*-pyrido[2,3-*b*]carbazole-5,11-dione (12a) and 6-Ethyl-9-hydroxy-4-methyl-6*H*-pyrido[3,2-*b*]carbazole-5,11-dione (12b): A solution of azadiene **10b** (0.280 g, 2.5 mmol) in dry toluene (20 mL) was added to a solution of carbazolequinone **9** (0.241 g, 1 mmol) in toluene (20 mL). The reaction mixture was heated to reflux for 1 h, then basic alumina (1 g) was added and the reflux maintained for a further 12 h. The alumina was then separated by filtration and washed three times with a mixture of CH_2Cl_2 and MeOH (50:50). After removing the solvents, the two regioisomers **12a** and **12b** were separated by column chromatography on silica gel with EtOAc as the eluent. They were obtained in 72% overall yield (0.220 g).

12a: Major product. M.p. 280 °C. IR (KBr): $\tilde{\nu}$ = 3370, 1655, 1640 cm^{-1}. ^1H NMR ([D$_6$]DMSO): δ = 1.38 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3), 2.82 (s, 3 H, 4-CH_3), 4.68 (q, *J* = 7.0 Hz, 2 H, CH_2CH_3), 7.04 (dd, *J* = 8.8, 2.2 Hz, 1 H, 8-H), 7.59 (d, *J* = 5.1 Hz, 1 H, 3-H), 7.63 (d, *J* = 2.2 Hz, 1 H, 6-H), 7.64 (d, *J* = 8.8 Hz, 1 H, 9-H), 8.75 (d, *J* = 5.1 Hz, 1 H, 2-H), 9.69 (s, 1 H, OH) ppm. HRMS: calcd. for $C_{18}H_{14}N_2O_3$ 306.10044; found 306.10047.

12b: Minor product. M.p. > 350 °C. IR (KBr): $\tilde{\nu}$ = 3280, 1635 cm^{-1}. ^1H NMR ([D$_6$]DMSO): δ = 1.38 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3), 2.80 (s, 3 H, 4-CH_3), 4.67 (q, *J* = 7.0 Hz, 2 H, CH_2CH_3), 7.02 (dd, *J* = 8.8, 2.0 Hz, 1 H, 8-H), 7.59 (d, *J* = 5.1 Hz, 1 H, 3-H), 7.64 (d, *J* = 2.0 Hz, 1 H, 10-H), 7.66 (d, *J* = 8.8 Hz, 1 H, 7-H), 8.76 (d, *J* = 5.1 Hz, 1 H, 2-H), 9.68 (s, 1 H, OH) ppm. HRMS: calcd. for $C_{18}H_{14}N_2O_3$ 306.10044; found 306.10048.

5,7,11-Triacetoxy-10-ethyl-3-methyl-10*H*-pyrido[2,3-*b*]carbazole (13): A mixture of pyridocarbazolequinone **11** (0.100 g, 0.33 mmol), zinc powder (0.360 g, 24 equiv.), sodium acetate (0.113 g, 6 equiv.) and acetic anhydride (40 mL) was stirred at room temperature for 45 min. Then, CH_2Cl_2 (150 mL) was added and the reaction mixture was filtered. After removing the solvent under vacuum, the residue was washed twice with a mixture of EtOAc and petroleum ether (50:50) and then dissolved in CH_2Cl_2 (10 mL). After filtration and evaporation of the solvent, compound **13** was obtained in 55% yield (0.078 g) as a yellow-greenish powder which was not stable under recrystallization conditions. M.p. 210 °C. IR (KBr): $\tilde{\nu}$ = 1755 cm^{-1}. ^1H NMR ([D$_6$]DMSO): δ = 1.50 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3), 2.38 (s, 3 H, 7-OCOCH$_3$), 2.54 (d, *J* = 0.8 Hz, 3 H, 3-CH_3), 2.62 (s, 3 H, 11-OCOCH$_3$), 4.52 (q, *J* = 7.0 Hz, 2 H, CH_2CH_3), 2.68 (s, 3 H, 5-OCOCH$_3$), 7.30 (dd, *J* = 8.5, 2.2 Hz, 1 H, 8-H), 7.36 (d, *J* = 8.5 Hz, 1 H, 9-H), 7.80 (d, *J* = 2.2 Hz, 1 H, 6-H), 7.96 (m, 1 H, 4-H), 8.78 (d, *J* = 2.2 Hz, 1 H, 2-H) ppm. HRMS: calcd. for $C_{24}H_{22}N_2O_6$ 434.14778; found 434.14778.

5,7,11-Triacetoxy-10-ethyl-4-methyl-10*H*-pyrido[2,3-*b*]carbazole (14): Compound **14** was prepared as above from **12a**. The reaction mixture was stirred at room temperature for 3 h. Since the crude product is not stable under recrystallization conditions, it was washed with acetone and then isolated in 45% yield (0.065 g). Yellow powder; m.p. 210 °C. IR (KBr): $\tilde{\nu}$ = 1755 cm^{-1}. ^1H NMR ([D$_6$]DMSO, 500 MHz): δ = 1.39 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3), 2.35 (s, 3 H, 7-OCOCH$_3$), 2.58 (s, 3 H, 11-OCOCH$_3$), 2.70 (s, 3 H, 5-OCOCH$_3$), 2.86 (s, 3 H, 4-CH_3), 4.59 (q, *J* = 7.0 Hz, 2 H, CH_2CH_3), 7.33 (d, *J* = 4.5 Hz, 1 H, 3-H), 7.43 (dd, *J* = 8.5, 1.5 Hz, 1 H, 8-H), 7.76 (d, *J* = 8.5 Hz, 1 H, 9-H), 7.78 (d, *J* = 1.5 Hz, 1 H, 6-H), 8.76 (d, *J* = 4.5 Hz, 1 H, 2-H) ppm. HRMS: calcd. for $C_{24}H_{22}N_2O_6$ 434.14778; found 434.14773.

12-Ethyl-9-hydroxy-7*H*-[2,7]naphthyridin[1,8a,8-*ab*]carbazol-7-one (15) and 12-ethyl-9-methoxy-7*H*-[2,7]naphthyridin[1,8a,8-*ab*]carbazol-7-one (16): *N*,*N*-Dimethylformamide dimethylacetal (0.165 g,

1.48 mL) was added dropwise under nitrogen to a solution of pyridocarbazolequinone **12b** (0.095 g,0.31 mmol) in DMF (1 mL). The reaction mixture was stirred and heated at 110 °C for 3 h. Then, ammonium chloride (0.030 g, 0.56 mmol) and acetic acid (1 mL) were added and the mixture was stirred and heated under nitrogen for 1 h. After the usual workup with dichloromethane, the organic phase was washed twice with a saturated solution of sodium bicarbonate and then with water. After removing the solvent, compounds **15** and **16** were separated by column chromatography on silica gel with a mixture of methanol and dichloromethane (6:100) as the eluent. The title compounds were obtained in 20% (0.020 g) and 30% (0.031 g) yield, respectively.

15: M.p. 258 °C. IR (KBr): $\tilde{\nu}$ = 3240, 1640 cm^{-1}. ^1H NMR ([D_6]DMSO): δ = 1.41 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3), 5.0 (q, *J* = 7.0 Hz, 2 H, CH_2CH_3), 6.91 (dd, *J* = 9.0, 2.0 Hz, 1 H, 10-H), 7.56 (d, *J* = 9.0 Hz, 1 H, 11-H), 7.68 (d, *J* = 2.0 Hz, 1 H, 8-H), 7.92 (d, *J* = 5.5 Hz, 1 H, 4-H or 3-H), 8.10 (d, *J* = 5.5 Hz, 1 H, 3-H or 4-H), 8.84 (d, *J* = 5.5 Hz, 1 H, 5-H or 2-H), 9.01 (d, *J* = 5.5 Hz, 1 H, 2-H or 5-H), 9.50 (s, 1 H, OH) ppm. HRMS: calcd. for $C_{19}H_{14}N_3O_2$ [M + 1] 316.1086; found 316.1088.

16: M.p. > 300 °C. IR (KBr): $\tilde{\nu}$ = 1620 cm^{-1}. ^1H NMR ([D_6]DMSO): δ = 1.42 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3), 3.87 (s, 3 H, OCH_3), 5.02 (q, *J* = 7.0 Hz, 2 H, CH_2CH_3), 7.02 (dd, *J* = 9.0, 2.0 Hz, 1 H, 10-H), 7.66 (d, *J* = 9.0 Hz, 1 H, 11-H), 7.71 (d, 1 H, *J* = 2. Hz, 8-H), 7.95 (d, *J* = 5.5 Hz, 1 H, 4-H or 3-H), 8.12 (d, *J* = 5.5 Hz, 1 H, 3-H or 4-H), 8.86 (d, *J* = 5.5 Hz, 1 H, 5-H or 2-H), 9.04 (d, *J* = 5.5 Hz, 1 H, 2-H or 5-H) ppm. HRMS: calcd. for $C_{20}H_{15}N_3O_2$ 329.1164; found 329.1162.

Inhibition Assays of *Toxoplasma gondii* Purine Nucleotide Phosphorylase: Purine nucleoside phosphorylase was isolated from two strains of *Toxoplasma gondii*, a virulent strain RH and a cystic strain ME 49. Both strains were maintained in culture with the human myelomonocytic cell line THP-1.[2] PNP was purified by the followings steps: precipitation with $(NH_4)_2SO_4$, DEAE-Sephacel and hydroxyapatite columns. The amount of the protein was determined by Bensadoun's method.[17] The drugs were tested on each PNP at doses of 1 and 4 mM. The inhibition percentage of PNP specific activity[3] was defined as the ratio of PNP specific activity with drug to PNP specific activity without drug. K_i values were determined by the Lineweaver−Burk method with inosine as the variable substrate ranging from 0.5 to 3 mM and fixed inorganic phosphate concentration (50 mM).

[1] For a review of carbazolequinones see: [1a] Z. Bouaziz, P. Nebois, A. Poumaroux, H. Fillion, *Heterocycles* **2000**, *52*, 977−1000. [1b] A. Poumaroux, Z. Bouaziz, M. Domard, H. Fillion, *Heterocycles* **1997**, *45*, 585−596. [1c] A. Poumaroux, Z. Bouaziz, H. Fillion, M. Domard, J. Giraud, A.-F. Pétavy, *Chem. Pharm. Bull.* **1999**, *47*, 643−646.

[2] P. Nebois, M.-E. Sarciron, B. Bibal, B. Bouammali, O. Cherkaoui, F. Pautet, A.-F. Pétavy, N. Walchshofer, H. Fillion, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 871−873.

[3] A. Ghérardi, M.-E. Sarciron, A.-F. Pétavy, F. Peyron, *Life Sci.* **1999**, *65*, 1733−1738.

[4] A. Bzowska, E. Kulikowska, D. Shugar, *Pharmacol. Therapeutic.* **2000**, *88*, 349−425.

[5] Diquinone **8** was obtained previously by oxidation of hydroquinone with oxygen in the presence of bis(1,3-propanediaminato)copper(II) chloride according to the procedure described in: S. M. Paraskevas, D. Konstantinidis, G. Vassilara, *Synthesis* **1998**, 897−899.

[6] C. Grundmann, *Ber. Dtsch. Chem. Ges.* **1936**, *69*, 1755−1757.

[7] [7a] α,β-Unsaturated *N*,*N*-dimethylhdrazones were first used as heterodienes in the Diels−Alder reaction in: B. Serckx-Poncin, A.-M. Hesbain-Frisque, L. Ghosez, *Tetrahedron Lett.* **1982**, *23*, 3261−3264. [7b] Formation of a hydroquinone tautomer was first reported in: K. T. Potts, E. B. Walsh, D. Bhattachargie, *J. Org. Chem.* **1987**, *52*, 2285−2292. [7c] For a recent review on cycloadditions of such azadienes, see: F. Pautet, P. Nebois, Z. Bouaziz, H. Fillion, *Heterocycles* **2001**, *54*, 1095−1138 and references therein.

[8] For the preparation of **10a**, see: A. Waldner, *Helv. Chim. Acta* **1988**, *71*, 486−492.

[9] For the preparation of **10b**, see: T. Severin, G. Wanninger, H. Lerche, *Chem. Ber.* **1984**, *117*, 2875−2885.

[10] For analogous dihydro derivatives observed in the cycloadditions of **10b** with naphthoquinones, quinoline or isoquinoline diones see:

[10a] M. Chigr, H. Fillion, A. Rougny, *Tetrahedron Lett.* **1988**, *29*, 5913−5916. [10b] Z. Bouaziz, P. Nebois, H. Fillion, J. L. Luche, G. Jenner, *Tetrahedron* **1995**, *51*, 4057−4064.

[11] By the use of pulsed field gradients, this new method allows to detect very small NOE enhancements and with complete confidence. For publications on this subject, see:

[11a] J. Stonehouse, P. Adell, J. Keeler, A. J. Shaka, *J. Am. Chem. Soc.* **1994**, *116*, 6037−6038. [11b] K. Stott, J. Stonehouse, J. Keeler, T.-L. Hwang, A. J. Shaka, *J. Am. Chem. Soc.* **1995**, *117*, 4199−4200.

[12] F. Bracher, *Liebigs Ann. Chem.* **1989**, 87−88.

[13] For a review on the use of formamide acetals as alkylating agents see: R. F. Abdulla, R. S. Brinkmeyer, *Tetrahedron* **1979**, *35*, 1675−1735.

[14] P. E. Daddona, W. P. Wiesmann, W. Milhouse, J. W. Chern, L. B. Townsend, M. S. Hershfield, H. K. Webster, *J. Biol. Chem.* **1986**, *261*, 11667−11673.

[15] A. Bzowska, E. Kulikowska, D. Shugar, *Biochim. Biophys. Acta* **1992**, *1120*, 239−247.

[16] C. M. Schimandle, L. Tanigoshi, L. A. Mole, I. W. Sherman, *J. Biol. Chem.* **1985**, *261*, 11667−11673.

[17] A. Bensadoun, D. Weinstein, *Anal. Biochem.* **1976**, 241−250.

Received November 9, 2001
[O01501]