SYNTHESIS AND PROTOZOOCIDAL ACTIVITY OF NEW 1,4-NAPHTHOQUINONES

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Abstract : Four naphthoquinones were submitted to the action of diazomethane. The structure of the nine adducts, of which four were original, was determined. The protozoocidal activity of these compounds was evaluated *in vitro* against *Plasmodium falciparum*, *Leishmania donovani*, *Trypanosoma brucei* and *Trichomonas vaginalis*. The 2-methoxy-naphthoquinone **3a** exhibited some activity and was more potent against these four protozoa than the reference drugs. The 2-methoxynaphthoquinone **4a** showed more activity than chloroquine towards *Plasmodium falciparum*.

Introduction

In our previous studies on the reactivity of diazomethane towards cyano derivatives, we showed that diazomethane could react with aromatic or heterocyclic compounds, such as chromones, bearing electroattractive groups (1,2,3).

In an extension of these studies, we reacted diazomethane with carbonyl compounds similar to benzopyrones: 1,4-naphthoquinone **1**, 2-methyl-1,4-naphthoquinone or menadione **2**, 2-hydroxy-1,4-naphthoquinone or lawsone **3** and 2-hydroxy-3-(E-4-parachlorophenylcyclohexyl)-1,4-naphthoquinone or atovaquone **4** (Wellvone™) used therapeutically. We chose unsubstituted, mono- or disubstituted naphthoquinones in attempt to investigate the difference of reactivity towards diazomethane as a function of the level of substitution and the nature of substituents.

We determined the antimalarial activity of the resulting adducts against *Plasmodium falciparum*. In this respect, several 2-hydroxynaphthoquinones have been described with antimalarial activity, and the quinones are known to interfere with coenzyme Q in the respiratory chain of some protozoa (4). We also studied the activity of these compounds towards flagellate protozoa: *Trypanosoma brucei* and *Leishmania donovani* in an attempt to enlarge the protozoocidal activity spectrum, as recent studies (5,6,7) have shown that naphthoquinones possess activity towards *Trichomonas vaginalis*.

Results and Discussion

Chemistry

The naphthoquinones **1**, **2**, **3** and **4** were stirred during several days with a solution of diazomethane in diethyl ether. The solvent was evaporated and the residue was chromatographed on a silica gel column (70-230 mesh). The structures were determined using spectroscopic methods (NMR ^{1}H, ^{13}C, 2D) and by X-ray diffraction.

Addition of diazomethane to 1,4-naphthoquinone **1** gave rise to two N-methylated isomers: **1a** (8) and 1b, which is an original compound.

These products were assumed to be formed after oxidation of the intermediate pyrazoline and methylation of the two prototropic forms of pyrazole (Figure 1). The pyrazoline and pyrazole were not isolated.

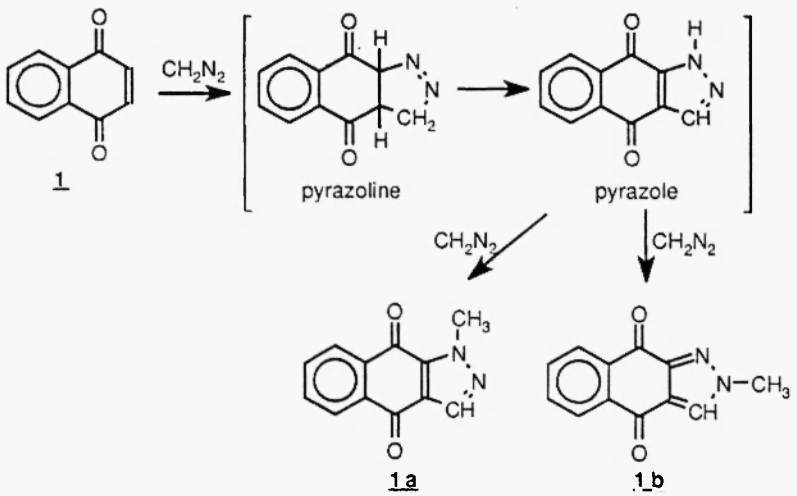


Figure 1

2-Methyl naphthoquinone 2 was added to a stoichiometric quantity of diazomethane solution, and gave a fused pyrazoline derivative 2a, not oxidizable into pyrazole. The decomposition of the adduct 2a gave rise to a cyclopropanic compound **2b** and 2,3-dimethyl-1,4-naphthoquinone **2c** as described by Dean (9,10) (Figure 2).

On the other hand, with an excess of diazomethane, we also isolated the compound **2c**, and an original product, the spiro-[3-cyclopropane]-2-hydroxy-2-methyl-2,3-dihydro-1,4-naphthoquinone **2d** (Figure 2) whose structure was verified by X-ray diffraction (Figure 3).

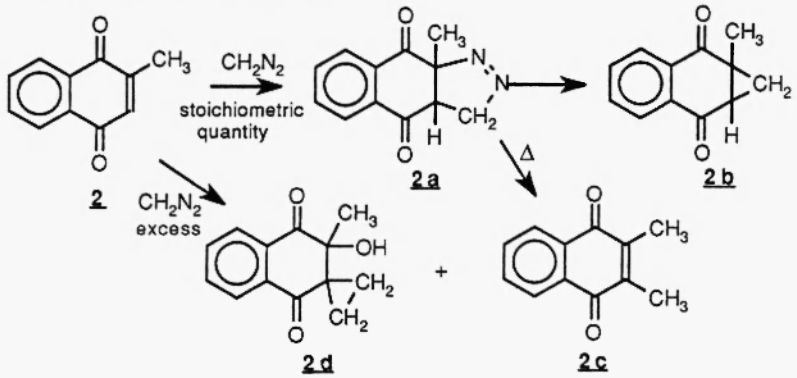


Figure 2

With lawsone 3, we obtained as usual the O-methylated compound **3a** (11) and the adduct **3b**, produced by a dipolar cycloaddition of diazomethane on **3a** (Figure 4).

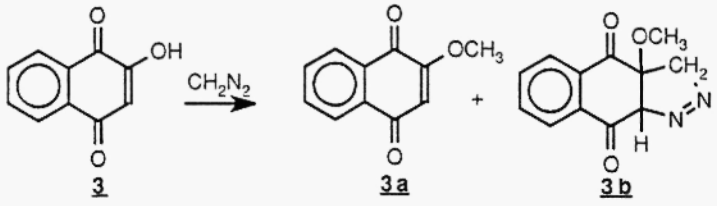
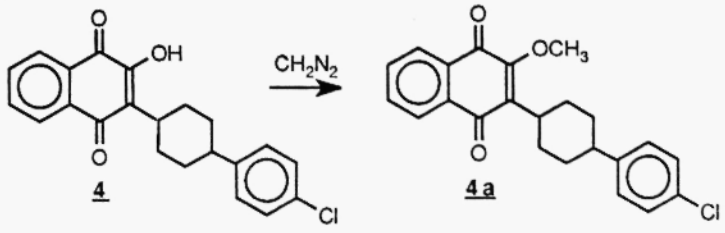


Figure 4

With atovaquone **4**, we only isolated the methoxylated compound **4a** (Figure 5).



4 **4a**

Figure 5

In the case of compounds **2a** and **3b**, diazomethane added to the double bond with opposite regiospecificity. Indeed, the ^1H NMR spectrum of **2a** displayed a triplet at δ 3.07 ppm assignable to the H_{3a} proton and a doublet at δ 4.98 ppm attributed to the CH_2 group, whereas the ^1H NMR spectrum of **3b** revealed a singlet at δ 5.92 ppm assigned to the H_{9a} proton and two doublets at δ 3.22 ppm and 3.61 ppm attributed to the CH_2 protons.

The difference in reactivity between these two compounds can be explained by the calculation of the atomic charges with the AM1 Hamiltonian (12). In the cycloaddition reactions, diazomethane usually reacts as the 1-3 dipole : $^-CH_2$-N=N$^+$. The AM1-calculated net atomic charges of carbon and nitrogen atoms are -0.283 and -0.05 respectively. In the case of the 2-methoxynaphthoquinone **3a**, the charges carried by the C_2 and C_3 atoms are +0.056 and -0.299 respectively. These values are in agreement with the experimentally observed orientation (compound **3b**). On the other hand, the charges carried by the C_2 and C_3 atoms of the 2-methylnaphthoquinone **2**, are -0.09 and -0.178 respectively. These values are not in favour of the experimentally observed orientation (**2a**). Nevertheless, the charge difference between C2 and C3 is less significant than that of 2-methoxynaphthoquinone. For methylnaphthoquinone both charges are negative and generate an overall attractive potential along the C2-C3 double bond, and so the cycloaddition in the latter case is probably governed by steric effects.

Pharmacology

In vitro activities against *Plasmodium falciparum, Leishmania donovani, Trypanosoma brucei* and *Trichomonas vaginalis* were determined using chloroquine, quinine, pentamidine and metronidazole as reference compounds. The results of the biological evaluation are expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth and are listed in table 1.

Plasmodium falciparum : activity levels ranged from 0.75 nM for atovaquone **4** to 150 μM for lawsone **3**. The efficiencies of **2a** and **4a** were comparable to that of chloroquine, the reference drug. Methylation led to a higher activity with lawsone but, in contrast, decreased that of atovaquone. However, this compound **4a** has a IC_{50} of 32 nM.

Leishmania donovani : the unsubstituted **1** or monosubstituted quinones **2** and **3a** exhibited the highest activity. Compounds **1** and **2** were more effective than pentamidine, the reference drug.

Trypanosoma brucei : only three compounds **2**, **3a** and **3b** were slightly active, with levels ranging from 12.5 μM to 25 μM, far less than that of the reference compound, pentamidine, 3 μM.

Trichomonas vaginalis : the activity of compound **3** was comparable to that of metronidazole. Methylation enhanced the trichomonacidal properties and **3a** was more active than the reference compound.

Table 1 :

Compounds	IC_{50}, µM *P. falciparum*	IC_{50}, µM *L. donovani* DD8	MEC, µM *T. brucei* GVR	IC_{50}, µM *T.vaginalis* CMP
1	16	0.9	50	>100
1a	30	>100	>100	96.1
1b	110	80	>100	80.6
2	4	1.2	25	>100
2a	0.85	20.2	>100	>100
2c	10,5	>100	>100	>100
2d	100	>100	>100	>100
3	150	>100	>100	50
3a	1.7	11.5	12.5	12.5
3b	1.4	40	25	>100
atovaquone 4	$0.75.10^{-3}$	50.5	>100	>100
4a	0.032	>100	>100	>100
chloroquine	0.125	-	-	-
quinine	0.18	-	-	-
pentamidine	-	7.7	3	
metronidazole	-	-	-	25.5

IC_{50} = Inhibitory concentration 50%.
MEC = Minimal effective concentration (minimal concentration that killed all the parasites).

The 2-methoxy-naphthoquinone **3a** was slightly active against all the tested protozoa, whereas its unmethylated precursor **3** was completely inactive. It was noteworthy that compounds **1**, **2** and **3b** were slightly active against *Plasmodium falciparum*, *Leishmania donovani* and *Trypanosoma brucei*, but devoid of activity against *Trichomonas vaginalis*, a protozoan with an anaerobic metabolism. This could be accounted for by the fact that the latter parasite harbours hydrogenosomes instead of mitochondria; naphthoquinones (*e.g.* atovaquone) are known to inhibit respiration processes but not in hydrogenosomes.

The double substitution in the 2 and 3 position of the quinones (compounds **4** and **4a**) enhanced the antimalarial activity, but nullified all the other protozoocidal properties.

Experimental

1- Chemistry

General procedure :

To a solution of diazomethane (5.5 g, 0.13 mole) in diethyl ether (600 ml), prepared according to Boer's method (13) was added the naphthoquinone (5.1 g, 0.03 mole). The mixture was maintained at room temperature for a week.The solvent was evaporated and the residue was chromatographed on a silica gel column (70-230 mesh).

1-Methyl-1H-benz [f] indazole-4,9-dione 1a : Mp = 176°C. (Litt. (8): 179°C).

2-Methyl-2H-benz [f] indazole-4,9-dione 1b : $C_{12}H_8N_2O_2$. M=212.20. Mp = 314°C. Yield = 40 %. MS (m/z): 212 (M^+). IR (KBr, cm^{-1}) : 3104, 2931 (CH, CH_3); 1662 (C=O).

^{1}H NMR ($CDCl_3$, δ ppm) : 4.12 (s, 3H, CH_3); 7.73 - 7.77 (m, 2H, H-6 and H-7); 8.04 (s, 1H, H-3); 8.23 (m, 1H, H-8); 8.31 (m, 1H, H-5). ^{13}C NMR (DMSO-d_6, δ ppm) : 39.09 (CH_3); 122.19 (C-3a); 126.71 and 126.90 (C-5 and C-8); 133.08 (C-3); 134.01 and 134.29 (C-4a and C-8a); 134.08 and 134.38 (C-6 and C-7); 147.35 (C-9a); 177.99 and 178.67 (C-4 and C-9). Anal. calc. for $C_{12}H_8N_2O_2$: C 65.98, H 4.03, N 14.00; found : C 66.01, H 4.16, N 13.95.

3a,9a-Dihydro-9a-methyl-3H-benz [f] indazole-4,9-dione 2a : Mp = 110°C. dec. (Litt. (9): 111°C). ^{1}H NMR ($CDCl_3$, δ ppm) : 1.65 (s, 3H, CH_3); 3.07 (t, 1H, H-3a); 4.98 (d,2H, CH_2); 7.72 (m, 2H, H-6 and H-7); 7.91 and 8.07 (m, 2H, H-5 and H-8).

1a,7a-Dihydro-1a-methyl-1H-cyclopropa [b] naphthalene-2,7-dione 2b: Mp = 65.5°C. (Litt.(9): 68°C).

2,3-Dimethyl-1,4-naphthoquinone 2c : Mp = 126°C. (Litt. (9): 123°C).

Spiro-[3-cyclopropane]-2-hydroxy-2-methyl-2,3-dihydro-1,4-naphthoquinone 2d : $C_{13}H_{12}O_3$. M = 216.23. Yield = 35 %. Mp = 88.5°C. MS (m/z): 216.08 (M^+). IR (KBr, cm^{-1}) : 3502 (OH); 3020, 2975 (CH, CH_2, CH_3); 1683,1675 (CO). ^{1}H NMR ($CDCl_3$, δ ppm) : 1.44 (s, 3H, CH_3); 0.75 (m, 1H, CH_2); 1.13 (m, 1H, CH_2); 1.39 (m, 1H, CH_2); 1.82 (m, 1H, CH_2); 3.81 (s, 1H, OH); 7.77 (m, 2H, H-6 and H-7); 8.08 (m, 2H, H-5 and H-8). ^{13}C NMR ($CDCl_3$, δ ppm) : 8.24 (CH_2); 17.57 (CH_2); 28.13 (CH_3); 38.39 (C-3); 74.77 (C-2); 126.75 and 127.11 (C-5 and C-8); 132.39 (C-8a); 134.63 and 134.97 (C-6 and C-7); 134.74 (C-4a); 194.98 (C-1); 201.79 (C-4).

2-Methoxy-1,4-naphthoquinone 3a : Mp = 185.5°C. (Litt. (11): 183.5°C).

3a,9a-Dihydro-3a-methoxy-3H-benz [f] indazole-4,9-dione 3b : $C_{12}H_{10}N_2O_3$. M = 230.22. Yield = 7%. Mp = 147.5°C. MS (m/z): 230.07 (M^+). IR (KBr, cm^{-1}) : 3060, 3995, 2850 (CH, CH_2, CH_3); 1644 (CO); 1601, 1611 (C=C). ^{1}H NMR ($CDCl_3$, δ ppm) : 3.22 and 3.61 (2d, 2H, CH_2, J = 8 Hz); 3.76 (s, 3H, CH_3); 5.92 (s, 1H, H-9a); 7.16 (d, 1H, H-5, J = 8Hz); 7.45 (m, 2H, H_6 and H_7); 8.06 (d, 1H, H_8, J = 5Hz);
^{13}C NMR ($CDCl_3$, δ ppm) : 52.41 (C-3a); 56.26 (CH_3); 57.88 (CH_2); 105.98 (C-9a); 122.73 (C-6 or C-7); 126.32 (C-5 or C-8); 128.53 (C-5 or C-8); 132.52 (C-5a); 132.57 (C-6 or C-7); 136.95 (C-8a); 169.09 and 184.43 (2CO).
Anal. calc. for $C_{12}H_{10}N_2O_3$: C 62.6, H 4.38, N 12.17; found : C 62.72, H 4.35, N 12.21.

2-Methoxy-3-(E-4-parachlorophenylcyclohexyl)-1,4-naphthoquinone 4a: $C_{23}H_{21}ClO_3$.
M= 380.87. Yield = 79%. Mp = 131°C. MS (m/z): 380,12 (M^+). IR (KBr, ν cm^{-1}) : 2941 and 2921 (CH, CH_2, CH_3); 1665 (CO). ^{1}H NMR ($CDCl_3$, δ ppm) : 1.46-2.19 (4m, 8H, 4CH_2 cyclohexyl), 2.61 and 3.18 (2m, 2H, 2CH cyclohexyl); 4.09 (s, 3H, CH_3); 7.16 (m, 2H, H-16 and H-20); 7.25 (m, 2H, H-17 and H-19); 7.67 (m, 2H, H-6 and H-7); 8.04 (m, 2H, H-5 and H-8). ^{13}C NMR ($CDCl_3$, δ ppm) : 29.89 and 34.42 (4CH_2); 35.18 and 43.27 (2CH); 61.31 (CH_3); 125.90 and 126.36 (2CH aro.); 128.18 and 128.40 (2CH ar.); 131.39 (C ar.); 131.45 (C-2); 132.23 (C ar.); 133.14 and 133.79 (2CH ar.); 138.85 (C ar.); 145.96 (C ar.); 181.73 and 185.41 (2CO).
Anal. calc. for $C_{23}H_{21}ClO_3$: C 72.53, H 5.55. found : C 72.44, H 5.55

X-ray analysis of compound 2d
A colourless crystal of 2d compound was used for obtaining intensity data on a CAD4 Enraf Nonius diffractometer with Cu Kα radiation. The cell parameters were refined using 25 reflexions centered on the diffractometer. The reflexions used in the analysis were corrected for Lorentz and polarisation effects, and also for absorption using the ψ scan method (14).
The experimental data are given in Table 2 and Table 3. The structure was solved by direct method procedure of Shelx 90 (Sheldrick, 1990) (15). All full -matrix least- squares refinements in this analysis were performed with Shelxs 93 (Sheldrick, 1993) (16).

2- Pharmacology

The in vitro tests were carried out following the methods previously described by Desjardins (17) for *Plasmodium falciparum* , by Mossman (18) and Mbongo (19) for *Leishmania donovani* , by Loiseau for *Trypanosoma brucei* (20) and *Trichomonas vaginalis* (21).

Figure 3 : ORTEP view of **2d**

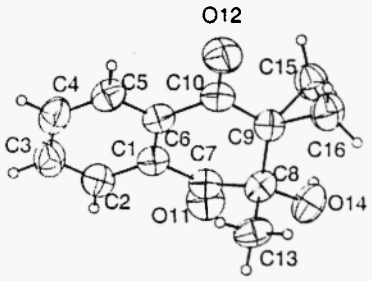


Table 2 : Crystal data and structure refinement for **2d**

$C_{13}H_{12}O_3$, MW=216.23, Triclinic, space group $\bar{P}$ 1,
a=7.294(4), b=8.979(4), c=9.077(8) Å,
α=95.57(5), β= 108.01(6), γ=107.16(5) °,
Z=2, V= 528.5(6) A^3, d_C=1.359 Mg/m^3,
μ=0.790 mm^{-1}, λ (Cu Kα) =1.54178A,
1772 independent reflexions (θmax=65°),
Refinement method : Full-matrix least-squares on F^2.
Final R indices ($I>2\sigma(I)$), R_1=0.0576, wR^2=0.1762,
S=1.123.

Table 3 : Positionnal parameters and mean recalculated isotropic for non-hydrogen atoms ($*10^{-4}$)

	x	y	z	U(eq)
C(1)	2961(3)	778(2)	3170(2)	44(1)
C(2)	1376(3)	-119(3)	3603(2)	56(1)
C(3)	992(4)	-1718(3)	3508(3)	65(1)
C(4)	2194(4)	-2445(3)	3032(3)	65(1)
C(5)	3774(3)	-1572(2)	2611(2)	57(1)
C(6)	4149(3)	39(2)	2639(2)	45(1)
C(7)	3377(3)	2496(2)	3260(2)	47(1)
C(8)	4253(3)	3203(2)	2070(2)	48(1)
C(9)	6096(3)	2677(2)	2145(2)	46(1)
C(10)	5842(3)	986(2)	2167(2)	48(1)
O(11)	3043(2)	3318(2)	4225(2)	64(1)
O(12)	7014(3)	385(2)	1862(2)	72(1)
C(13)	2606(4)	2575(3)	423(2)	60(1)
O(14)	4800(3)	4870(2)	2380(2)	66(1)
C(15)	8244(3)	3815(3)	3126(3)	64(1)
C(16)	7526(4)	3518(3)	1368(3)	67(1)

References

(1) G. Mouysset, G. Grassy, M. Payard, G. Commenges, A. Carpy, J. Couquelet, J. Heterocyclic Chem. 25, 1167 (1988).
(2) S. Danoun, G. Baziard-Mouysset, J.L. Stigliani, G. Commenges, A. Carpy, M. Payard, Bull. Soc. Chim. Fr. 132, 943 (1995).
(3) S. Danoun, G. Baziard-Mouysset, J.L. Stigliani, M. Payard, M. Selkti, B. Viossat, A. Tomas, Heterocyclic communications 4, 45 (1998).
(4) A. Bryskier, M.T. Labro, Paludisme et Medicaments, Arnette, Paris 1988, Chap VI, 171.
(5) C. Tournaire, R. Caujolle, M. Payard, G. Commenges, M. H. Bessières, C. Bones, P. M. Loiseau, P. Gayral, Eur. J. Med. Chem. 31, 507 (1996).
(6) S. Centonze, A. Bekaert, J.D. Brion, 5° Journée Jeunes Chercheurs, Société de Chimie Thérapeutique, Paris 16/01/1998.
(7) L. Salmon, V. Landry-Luca, C. Sergheraert, E. Davioud-Charvet, 5° Journée Jeunes Chercheurs, Société de Chimie Therapeutique, Paris 16/01/1998.
(8) B. Eister, H. Fink, A. Müller, Chem. Ber. 95, 2403, (1962).
(9) F.M. Dean, P.G. Jones, R.B. Morton, P. Sidisunthorn, J. Chem. Soc. 5336 (1963).
(10) F.M. Dean, L.E. Houghton, R.B. Morton, J. Chem. Soc. (C) 1980 (1967).
(11) R. H. Thomson in Naturally occuring quinones, London, Butter Worths Scientific publication, 1957.
(12) M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, J. Am. Chem. Soc. 107, 3902 (1985).
(13) T.J. Boer, H.J. Baker, Organic Syntheses 4, 251 (1963).
(14) A.C.T. North, D.C. Phillips, F.S. Mathews, Acta Cryst. A24, 351-359 (1990).
(15) G.M. Sheldrick, Acta Cryst. A46, 467-473 (1968).
(16) G.M. Sheldrick, (1993). Shelxs 93. Program for the refinement of crystal structures. University of Göttingen, Federal Republic of Germany.
(17) R.E. Desjardins, C.J. Canfield, J.D. Haynes J.D. and Chulay J.D., Antimicrob. Agents Chemother. 16, 710 (1979).
(18) R. Mossman, J. Immunol. Meth. 65, 55 (1983).
(19) N. Mbongo, P.M. Loiseau , F. Lawrence, C. Bories, D.G. Craciunescu, M. Robert-Gero, Parasitol. Res. 83, 515 (1997).
(20) P.M. Loiseau, C. Bories, M. Trabelsi, P. Gayral, J.G. Wolf, Parasitol. Res. 80, 708 (1994).
(21) P.M. Loiseau, C. Bories, A. Sanon, Drug Research (1998) in press.

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