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Synthesis and protozoocidal activities of quinones

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Introduction

Synthesis and pharmacological investigations of new quinones have been stimulated because of the antimalarial activity of some derivatives of lawsone (2-hydroxy-1,4-naphthoquinone) like menoctone [1], parvaquone [2, 3] and atovaquone [4, 5].

Chemistry

Condensation of acrolein with 2-hydroxy-1,4-naphthoquinone was performed under various experimental conditions (table I) to give **1** and **2**, previously described by Hayashi et al [6], and two new products **3** and **4**. The ethyl ether derivative **5** was directly obtained from **2** in acidic ethanolic medium; the yields are reported in table I.

Two reactional pathways could be considered for the condensation between acrolein and 2-hydroxy-1,4-naphthoquinone: a Claisen–Schmidt reaction or a Michael reaction, leading respectively to **1** or **2** after acetalizing (scheme 1). When the reaction was performed in the presence of an excess of hydrochloric acid (molar ratio HCl/lawsone >1), the synthesis of **1** and **2** gave two other products **3** and **4**, with yields less than 10% for each one.

Under these experimental conditions, very low yields were probably a result of the formation of polymeric by-products; nevertheless, the synthesis of two new compounds **3** and **4** was favored. The synthesis of **3** progressed presumably via the formation of a tricyclic intermediate followed by chlorination of a double bond; such reaction required the presence of Cl_2 in the media. As redox potentials of quinone are known [7], we may suppose that lawsone oxidized partly HCl into Cl_2. The formation of **4**, only obtained in hydrochloric media, could result from cyclization of the tautomeric form of the Michael adduct.

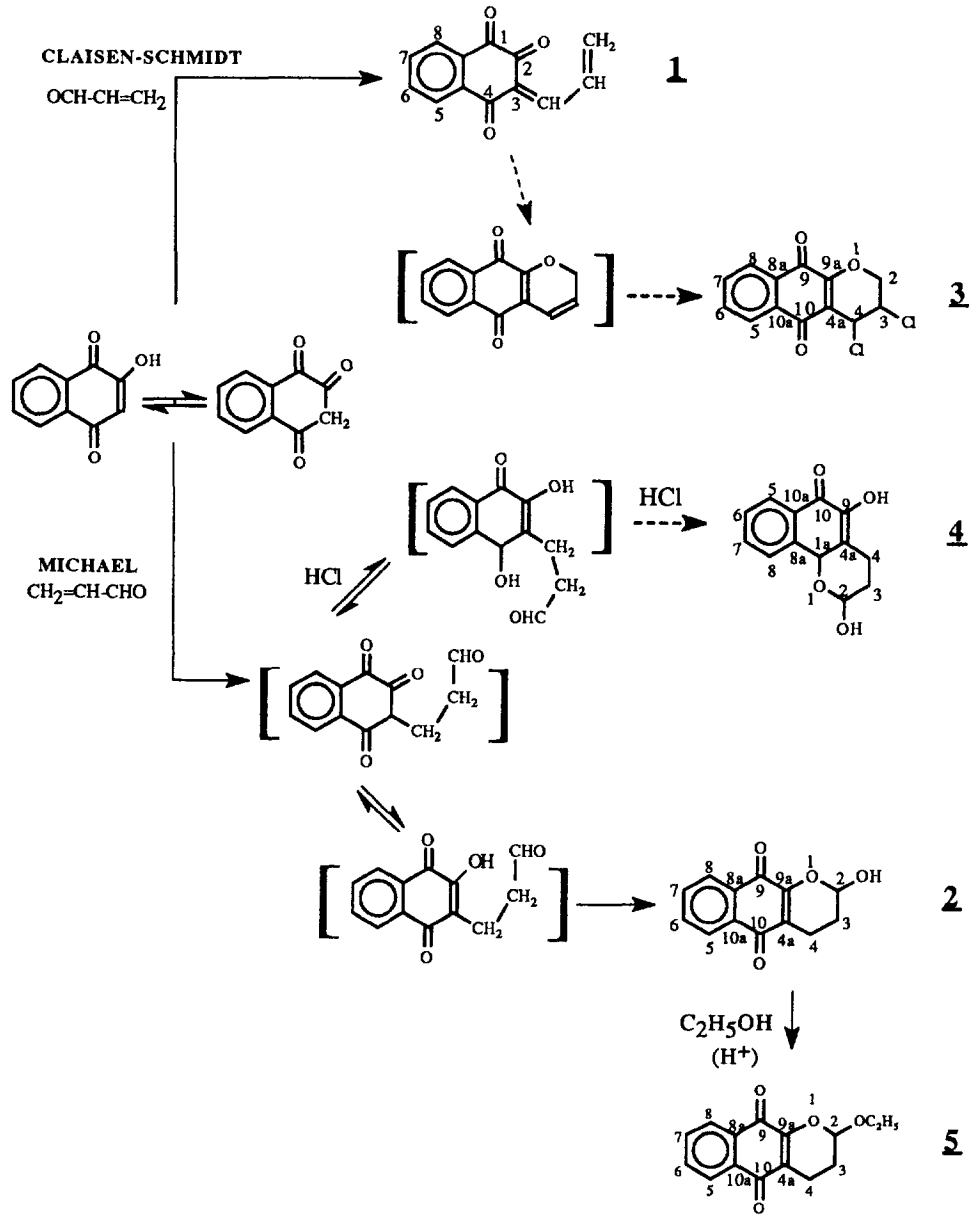
Table I. Yield of quinone isolement according to the HCl/lawsone ratio for condensation of acrolein with lawsone.

HCl/Lawsone ratio	*Yield (%)*			
	1	*2*	*3*	*4*
0[a]	33	6	–	–
0.4[a]	2.7	4.6	–	–
0.7[a]	5.4	–	3.4	–
2.1[a]	6	–	8	2.5
0[b]	20	15	–	–
0.4[b]	28	9.7	–	–
4.2[c]	12	13	1	2.5
2.1[d]	2	–	<1	9.7

[a]Acrolein added in one go (80 °C); [b]acrolein dropped (80 °C); [c]acrolein dropped, reflux of acetic acid (118 °C); [d]acrolein added in one go, reflux of acetic acid (118 °C).

By examination, from the NMR-^{1}H spectrum of **4**, of the coupling for the H_{1a} proton of each pair of eniantiomers, we found that the pair 2(*R*)1a(*S*) and 2(*S*)1a(*R*) was the more abundant. Actually, the signal recorded for the H_{1a} proton of the major product is a singlet (4.26 ppm) and by inspection of the Dreiding models we see that the H_{1a} of 2(*R*)1a(*S*)/2(*S*) 1a(*R*) isomers cannot be coupled; obviously, we may conclude that the pair 2(*R*)1a(*S*)/2(*S*)1a(*R*) is the major component of the mixture (75%). In contrast, the H_{1a} protons of the minor enantiomer pair 2(*R*)1a(*R*)/ 2(*S*)1a(*S*) (25%) may couple, and the ^{1}H-NMR signal is a multiplet at 4.05 ppm.

When performed without HCl, the reaction led only to **1** and **2**, with increased yields (33%) for **1** in comparison with those previously described (20%) in the presence of an unspecified amount of hydrochloric acid [6]; the formation of **2** was improved when the molar ratio HCl / lawsone was smaller than 0.5.



Scheme 1. Condensation of 2-hydroxynaphthoquinone with acrolein.

Condensation of lawsone wiyh propionaldehyde

When performed in hydrochloric media as described by Hooker [8], the reaction gave **6**; yields were higher than with acrolein. Furthermore, the formation of **7**, not reported by this route, might be explained by addition of a second molecule of propionaldehyde (scheme **2**). The structure of **7** was confirmed by comparison with an authentic sample [9].

Biological evaluation and discussion

Activities against *Toxoplasma gondii* and two species *of Leishmania* were determined in vitro. The MIC (minimum inhibitory concentration) values were determined against *T gondii*; sulfadiazine was used as reference. For *Leishmania*, IC_{50} and IC_{90} values were determined in vitro, using pentamidine as reference compound. The results of the biological evaluation are reported in table II.

Activity against *T gondii* in vitro was observed with all the synthesized compounds. MIC values for the quinones were in the same range of concentrations as sulfadiazine. An interesting result is the good activity found for dimethylanthraquinone **7** against *T gondii,* because activity of anthraquinones against *Toxoplasma* had not been reported to date. This interesting result obtained for the dimethyl derivative **7** led us to spread our study to the unsubstituted anthraquinone, but we found it inactive at the same dose (MIC > 38 μM).

Three compounds showed activities with IC_{50} values inferior to 50 μM against *Leishmania donovani and L major*. Among the compounds **3**, **5** and **1**, **1** is the most efficient against both *Leishmania* sp and *T gondii* although the metabolisms of these two parasites are very different. Its efficiency against *L donovani* is in the same range as pentamioline, used as reference compound.

The activity of the quinones decreased significantly when a hydroxy group in position 2 replaced the dichloro substitution at the 3- and 4-positions. However, the activity was enhanced when this hydroxy group was substituted with an ethyl moiety, perhaps due to its increased lipophilicity.

Toxoplasmosis and leishmaniasis, likewise, are opportunistic diseases in AIDS and prompt us to further investigations using both in vivo rodent leishmaniasis and toxoplasmosis models.

Experimental protocols

Chemistry

Condensation with acrolein

Lawsone (5 g, 0.028 mol, purchased from Aldrich Company) was dissolved in 35 mL acetic acid at 80 °C; acrolein (5 mL, 0.075 mol) was then dropped or added in one go, without HCl or just after addition of a defined quantity of HCl (see table I). The mixture was stirred for 2 h at 80 °C or to reflux in acetic acid medium and became very dark. After two hours, 200 mL water was added to the mixture; compounds **1**, **3** and **4** precipitated together and were separated by chromatography on a silica-gel column: **1** and **3** were first eluted with methylene chloride, then **4** with methylene chloride/ethyl acetate (80:20). Concentration of the aqueous layer gave **2**, which was purified on a silica-gel column with methylene chloride/ethyl acetate (90:10) as eluant. The ether **5** was obtained by heating **2** in ethanol and filtering after cooling.

In the absence of HCl, **3** and **4** were not synthesized and **1** precipitated alone. As shown in table I, yields were modified according to the experimental conditions.

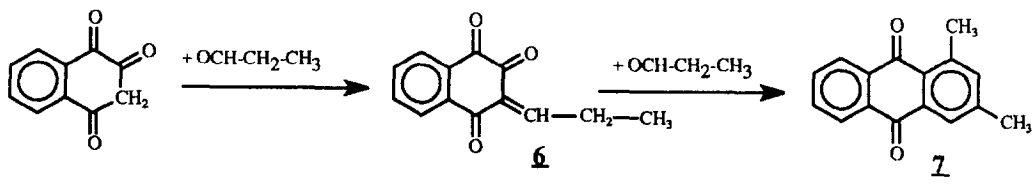
Condensation with propionaldehyde

Lawsone (5 g, 0.028 mol) was dissolved in acetic acid (35 mL); 5 mL of HCl were introduced immediately before addition of 12.5 mL (0.17 mol) propionaldehyde. The mixture was stirred at 80 °C for 1.5 h. Then 200 mL water was added, and the reaction medium became very dark. The mixture was kept for a further 3 h in the dark. After decantation and filtration, the brown oily residue was dissolved in diethyl ether and extracted by aqueous NaOH (10%). After acidification and extraction with petroleum ether, **6** was obtained and purified by recrystallization in petroleum ether (yield: 20%).

Evaporation of the diethyl ether layer led to **7**, which was purified by chromatography on a silica-gel column with cyclohexane/methylene chloride (80:20); yield: 12%.

Physicochemical data

Melting points were determined on a Kofler bank, and R_f values were measured after migration on silica plates (silica gel 60 HF_{254}) in methylene chloride or ethyl acetate. IR spectra (KBr pellets) were on a Perkin Elmer Model 983 G infrared spectrophotometer. NMR spectra were collected on a Brüker 200 spectrometer, except for the assignment of diastereoisomeric structures **4**. f2D Gradient-enhanced COSY (GE-COSY), gradient-enhanced HMQC and long range experiments (GE-HMQC) were recorded on a Brüker AMX 400 spectrometer equipped with a pulse field gradient accessory and data were processed with UX NMR (Brüker software package).



Scheme 2. Condensation of 2-hydroxynaphtoquinone with propionaldehyde.

Table II. In vitro activities against *T gondii* (MIC (μM)), *L major* and *L donovani* (IC_{50} and IC_{90} (μM)).

Compound	*Toxoplasma gondii MIC (μM)*	*Leishmania major*		*Leishmania donovani*	
		IC_{50} (μM) (day 8)	*IC_{90} (μM) (day 8)*	*IC_{50} (μM) (day 8)*	*IC_{90} (μM) (day 8)*
Sulfadiazine	16	–	–	–	–
Pentamidine	–	2	4.5	4	7
1	9	10	27	3	7
2	17	>500	>500	>500	>500
3	14	25	71	17	39
4	30	>500	>500	372	>500
5	11	27	48	21	37
6	23	>500	>500	>500	>500
7	16	>500	>500	>500	>500
Anthraquinone	>38	–	–	–	–

2-Oxo-3-(prop-2-ethylidène)-2,3-dihydro-1,4-naphthoquinone ***1*** *($C_{13}H_8O_3$).* Mp: 230 °C; mp = 140–145 °C was previously described for a mixture of *cis–trans* isomers [6].

2-Hydroxy-2H-3,4-dihydronaphtho[2,3-b]pyran-9,10-dione ***2*** *($C_{13}H_{10}O_4$).* Mp: 146 °C; lit: 146–147 °C [6].

3,4-Dichloro-2H-3,4-dihydronaphtho[2,3-b]pyran-9,10-dione ***3*** *($C_{13}H_8Cl_2O_3$).* Mp: 179 °C; R_f 0.67 (silica gel, CH_2Cl_2); IR (KBr), ν cm^{-1}: 1685, 1635, 1620 (C=O); 1595 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 4.56 (m, 1H, H_3), 4.63–4.89 (dt and dd, 2H, H_2), 5.27 (m, 1H, H_4), 7.77 (m, 2H, H_6 and H_7), 8.15 (m, 2H, H_5 and H_8); ^{13}C-NMR (DMSO-d_6) δ: 47.3 (C_3), 52.4 (C_4), 66.4 (C_2), 117.2 (C_{4a}); 126.7 and 126.8 (C_5 and C_8), 130.9 and 131.7 (C_{8a} and C_{10a}), 133.7 and 134.5 (C_6 and C_7), 153.9 (C_{9a}), 178.6 (C_{10}), 181.9 (C_9).

2,9-Dihydroxy-10-oxo-1a,10-dihydronaphtho[1,2-b]pyran-4 ***4*** *($C_{13}H_{12}O_4$).* Mixture of diastereoisomers; mp: 170 °C; R_f 0.88 (silica gel, EtOAc); IR (KBr) ν: 3405 (OH); 2958, 2935, 2903 (CH, CH_2); 1661 (C=0); 1597 cm^{-1} (C=C).

Isomers 2(R)1a(S) and 2(S)1a(R). ^{1}H-NMR (DMSO-d_6) δ: 1.20 and 1.78 (2m, 2H, H_3), 1.70 and 2.42 (2m, 2H, H_4), 3.66 (dd, 1H, H_2), 4.26 (s, 1H, H_{1a}), 5.73 (s, 1H, OH in 9)*, 6.29 (s, 1H, OH in 2)*, 7.52 (dd, 1H, H_8), 7.78 (m, 2H, H_6 and H_7), 7.81 (dd, 1H, H_5); ^{13}C-NMR (DMSO-d_6) δ: 27.4 (C_3), 37.3 (C_4), 71.8 (C_2); 73.2 (C_{1a}), 78.8 (C_{4a}), 125.5 and 125.8 (C_6 and C_7), 128.3 (C_8), 131.0 (C_{8a}), 135.9 (C_5), 145.7 (C_{10a}), 193.6 (C_9), 204.8 (C_{10}).

Isomers 2(S)1a(S) and 2(R)1a(R). ^{1}H-NMR (DMSO-d_6) δ: 1.03 and 1.94 (2m, 2H, H_3), 1.80 and 1.90 (2m, 2H, H_4), 3.76 (d, 1H, H_2), 4.05 (m, 1H, H_{1a}), 5.70 (s, OH in 9)*, 6.26 (s, OH in 2)*, 7.52 (dd, 1H, H_8), 7.78 (m, 2H, H_6 and H_7), 7.81 (dd, 1H, H_5); ^{13}C-NMR (DMSO-d_6) δ: 28.2 (C_3), 36.9 (C_4), 72.9 (C_2), 73.0 (C_{1a}), 78.4 (C_{4a}), 124.8 and 125.0 (C_6 and C_7), 128.2 (C_8), 132.6 (C_{8a}), 135. 6 (C_5), 145.4 (C_{10a}), 192.4 (C_9), 203.8 (C_{10}).

2-Ethoxy-2H-3,4-dihydronaphtho[2,3-b]pyran-9,10-dione ***5*** *($C_{15}H_{14}O_4$).* Mp: 135 °C; R_f 0.52 (silica gel, CH_2Cl_2); IR (KBr) ν cm^{-1}: 2880, 2850 (CH_3, CH_2); 1680, 1650, 1620 (C=O); 1597 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 1.21 (t, 3H, CH_3, J = 7 Hz), 1.77–2.20 (m, 2H, H_3), 2.49–2.77 (m, 2H, H_4), 3.66–4.02 (ddq, 2H, OCH_2CH_3, ^2J = 9.7 Hz; ^3J = 7.1 Hz), 5.49 (m, 1H, $CHOCH_2CH_3$), 7.69 (m, 2H, H_6 and H_7), 8.09 (2m, 2H, H_5 and H_8).

2-Hydroxy-3-(prop-1-enyl)-1,4-naphthoquinone ***6*** *($C_{13}H_{10}O_3$).* 100% *trans* isomer. Mp: 135–136 °C; lit: 135 °C [8].

*The disappearance of signals at 5.70, 5.73, 6.26 and 6.29 ppm on addition of CF_3COOD confirmed the attribution to OH groups.

1,3-Dimethyl-9,10-anthraquinone 7 ($C_{16}H_{12}O_2$). Mp: 160 °C; lit: 159–160 °C [9].

Pharmacological evaluation

In vitro determination of the anti-toxoplasma activity
In vitro studies were carried out with the virulent RH strain on MRC_5 fibroblast tissue cultures [10] by a method adapted from Derouin [11]. Toxoplasma growth was assessed by immunofluorescence performed directly on the fixed culture.

In vitro activity determination against Leishmania

L major MON 74 (MHOM/PT/92/CRE 26) and *L donovani* (MHOM/ET/67/L82; LV9) were maintained by monthly passage of amastigotes in hamsters. Promastigotes at stationary phase were suspended at a concentration of 5×10^5 per mL of culture medium and then 200 µL were deposited in each well of sterile 96-well plates (Nunc). The compound dissolved in dimethyl sulfoxide (DMSO) was added, the DMSO concentration staying less than 1%. Pentamidine isethionate was used as reference compound. Antileishmanial activity was assessed by MTT colorimetric assay [12] after 7 days of incubation (on day 8) and IC_{50} values, ie, concentrations inhibiting 50% parasite growth, were determined.

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