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Quinonic Derivatives Active Against a Virulent Strain of *Toxoplasma gondii*. Synthesis of 2-Methylfuro[2,3-*g*]- and [3,2-*g*]isoquinolinetriones

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Abstract—2-methylfuro[2,3-*g*]isoquinoline-4,7,9-trione (**4**) and 2-methylfuro[3,2-*g*]isoquinoline-4,6,9-trione (**5**) were prepared regiospecifically from 2-azadiene **9** and bromobenzofuran-4,7-diones **1** or **11**. The activity of these two compounds and some other quinonic derivatives was evaluated in vitro against a virulent strain of *Toxoplasma gondii*. Compounds **4** and **7** were found to be as active as pyrimethamine. © 2000 Elsevier Science Ltd. All rights reserved.

Toxoplasmosis is a common zoonosis asymptomatic in most adults, caused by the protozoan *Toxoplasma gondii*. However, this parasite can be involved in two important pathologies: congenital and encephalitic toxoplasmosis.[1] Congenital toxoplasmosis usually occurs as a result of primary maternal infection and can lead to abortion in the first three months of the pregnancy. When the foetus infection occurs later, this disease is characterized by predominant neurological and ophtalmologic manifestations. The percentage of transplacental transmission varies between countries. For example, studies in the United Arab Emirates[2] and France[3] have shown a transmission rate of 38 and 29%, respectively, with a variable occurrence of congenital toxoplasmosis related to the contamination date during the pregnancy. It seems that a treatment with the most effective drugs, like pyrimethamine, does not lead to a complete destruction of the parasite. This latter can develop cysts, which are mainly localized in the cerebral tissue. These cysts are reactivated in immunodeficient patients like AIDS patients, which leads to an encephalitic toxoplasmosis.[4]

We are engaged in a program towards the search of active drugs against *T. gondii*.[5] On the other hand, antiparasitic activity of naphthoquinones derivatives has been recognized; for instance, some of them are active against another protozoan *Trypanosoma cruzi*.[6] Considering our experience in the synthesis of heteropolycyclic quinones, we planned to synthetize and test furoisoquinolinetriones **4** and **5** against the virulent RH strain of *T. gondii*. Other quinonic derivatives: benzoisoquinolinetriones **3a**–**c**,[7] **3d**–**e**,[8] furoquinolinediones **6**–**7**,[9] and two synthetic intermediates **1**[9] and **2**[10] were also screened in order to explore structure–activity relationships (Fig. 1). In vitro biological studies have been performed using an infected human myelomonocytic cell line THP-1 and four well-known effective drugs as positive controls: sulfadiazine, clindamycine, pyrimethamine and atovaquone.

Synthesis of 2-methylfuro[2,3-*g*]isoquinoline-4,7,9-trione 4 and 2-methylfuro[3,2-*g*]isoquinoline-4,6,9-trione 5. The [4 + 2] cycloaddition between 2-azadiene **9**[11] and furoquinone **10**[9] ($CHCl_3$, 20 °C, 48 h), followed by an acidic hydrolysis (HF 40%, 20 °C, 2 h), led to an inseparable mixture of regioisomers **4** and **5**, in 40% overall yield (**4**/**5** = 38/62, observed from the ^1H NMR spectrum). The orientation of the regiochemistry, as well as

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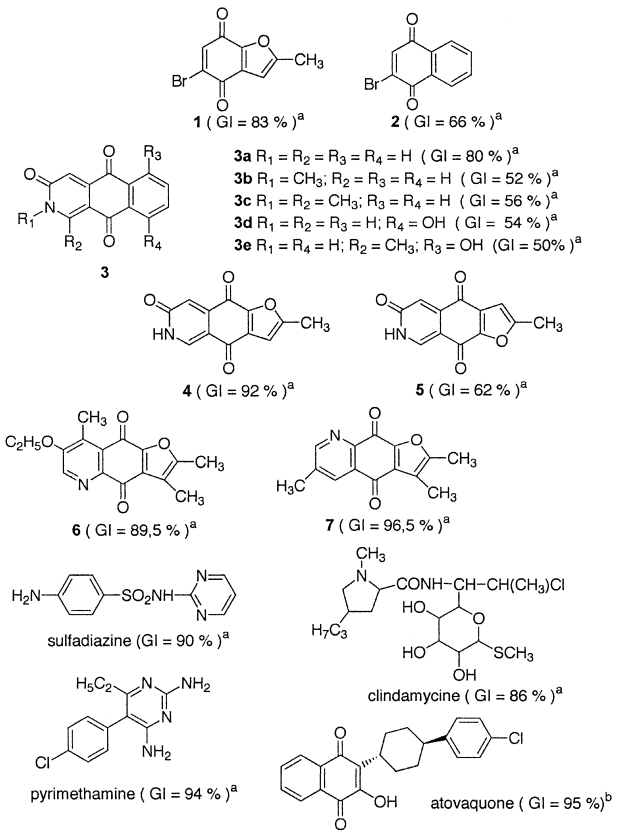
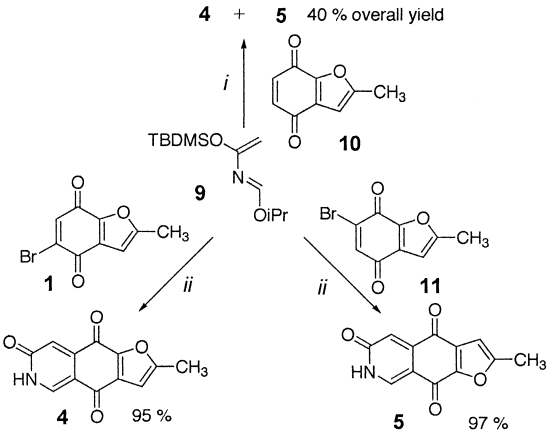


Figure 1. Structure and growth inhibition % (GI) of the compounds tested against *T. gondii* at the concentration of 2 μg/mL (a) or 0.1 μg/mL (b).

the poor regioselectivity, is in agreement with the molecular orbital frontier theory. Indeed, for azadiene **9**, the larger HOMO coefficient is located at C-4 (the values are 0.236 for C-1 and 0.691 for C-4)[12] while for quinone **10** that of the LUMO is situated at C-5, with a low difference between C-5 and C-6 (C-5 = 0.365, C-6 = 0.344).[9] With the aim to increase the regioselectivity and considering our precedent experiences in the field of brominated quinones[9,12,13] we then oriented this work to the use of furoquinones **1** and **11**.[9] Thus, reactions of azadiene **9** towards these dienophiles, carried out in $CHCl_3$ at room temperature for 72 h, gave regiospecifically the adducts **4** or **5**, respectively (Scheme 1). In this case, the acidic hydrolysis step was unnecessary because of the in situ formation of HBr. Moreover, the yields of these cycloadditions (95% from **1** and 97% from **11**) were dramatically improved compared to those obtained from the unbrominated quinone **10** (40%). The regiochemistry observed with furoquinones **1** and **11** is in contradiction with frontier molecular orbital theory (**1**: C-5 = 0.378, C-6 = 0.371 and **11**: C-5 = 0.390, C-6 = 0.358) but it can be explained by the well-known blocking effect of the bromine atom.[9]

Results and Discussion

Percentages of parasite growth inhibition in THP-1 cells by compounds **1**–**7** and controls are shown in Figure 1. The most active reference compound is atovaquone



Scheme 1. *i*: (1) $CHCl_3$, rt, 48 h, (2) HF 40%, 2 h, *ii*: $CHCl_3$, rt, 72h.

(GI = 95% at 0.1 μg/mL), but it is also known for its cytotoxicity.[14] Indeed, we have noted at this concentration a deep alteration of THP-1 cells. No cytotoxic effect has been observed for quinones **1**–**7** and the other reference compounds at 2 μg/mL. Quinones **1**–**7** possess significant antiparasitic activities at this concentration. Among benzoisoquinolinetriones **3**, the most simple product **3a** is also the most active (GI = 80%). The introduction of a phenolic OH moiety (**3a** versus **3d**) or *N*-methylation (**3a** versus **3b**) leads to a marked loss of activity (~30%), and the additional presence of a methyl group as R_2 has a negligible effect (**3b** versus **3c**). The nature of the fused ring in isoquinolinetrione derivatives **3**–**5** and in synthetic intermediates **1**–**2** affects the activity (**3a** versus **4**, **1** versus **2**). A furan fused ring enhances it, while the regioisomeric structure seems to have a significant influence (**4** versus **5**). Finally, two compounds, **4** and **7**, exhibit nearly equal inhibitory activity to pyrimethamine.

Conclusion

Quinonic derivatives **1**–**7** inhibit in different ranges the in vitro growth of *T. gondii*. The most active compounds **4** and **7** are benzofuran derivatives. This suggests that the furan ring plays a beneficial role on activity, but it is noteworthy that the regioisomeric structure seems to be of great importance. As part of our research program on drugs active in vivo against *T. gondii* brain cysts, compounds **4**, **7** and derivatives will be tested on mice.

Experimental Procedures

Synthesis

A solution of 3-*tert*-butyldimethylsilyloxy-1-isopropoxy-2-azabuta-1,3-diene **9**[11] (0.61 g, 2.5 mmol) in 3 mL of anhydrous $CHCl_3$ was slowly added to a solution of 5-bromo-2-methylbenzo[*b*]furan-4,7-dione **1** or 6-bromo-2-methylbenzo[*b*]furan-4,7-dione **11** (0.24 g, 1 mmol) in 3 mL of the same solvent under an argon atmosphere at room temperature. Then, the mixture was stirred for 72 h. Filtration afforded **4** (from **1**) or **5**

(from **11**) as a powder which was washed with 2x5 mL CH_2Cl_2/MeOH (95/5), then with 5 mL of diethyl ether. **4**: yellow powder, 95% yield; mp (Büchi 510) 205 °C (dec). IR (KBr) 3440, 1675, 1655 cm^{-1}. ^1H NMR (DMSO-d_6, δ ppm): 12.63 (bs, 1H, NH); 8.18 (s, 1H, H-5); 6.84 (s, 1H, H-8); 6.82 (d, 1H, J = 1Hz, H-3); 2.50 (CH_3-2, with DMSO). EI MS m/z (229, M^+). **5**: orange powder, 97% yield; mp (Büchi 510) > 300 °C. IR (KBr) 3440, 1675, 1655 cm^{-1}. ^1H NMR (DMSO-d_6, δ ppm): 12.69 (bs, 1H, NH); 8.23 (s, 1H, H-8); 6.83 (s, 1H, H-5); 6.82 (d, 1H, J = 1 Hz, H-3); 2.50 (CH_3-2, with DMSO). EI MS m/z (229, M^+).

Growth inhibition of *T. gondii*

The virulent RH strain of *T. gondii* was maintained in culture with the human myelomonocytic cell line THP-1 (ECACC number 88081201, Sophia-Antipolis, France) as previously described.[5] Three different experiments were performed in quadruplicates.

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