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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2773–2776

Synthesis of new fluoroquinolones and evaluation of their in vitro activity on *Toxoplasma gondii* and *Plasmodium* spp.

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Received 28 January 2004; revised 19 March 2004; accepted 24 March 2004

Abstract—The synthesis of four new computer-designed fluoroquinolones which have been predicted by QSAR analysis to be active against the protozoa *Toxoplasma gondii* is described. These compounds are inhibitory in vitro for *T. gondii.* One of these compounds has a remarkably high activity comparable to that of trovafloxacin. It combines the basic cyclopropyl–quinoline structure of gatifloxacin or moxifloxacin with the C-7 6-amino-3-azabicyclo[3.1.0]hexyl side chain of trovafloxacin. The four compounds are also inhibitory for blood stages of *Plasmodium falciparum* though at high concentration. These results confirm the potential of quinolones as *anti-T. gondii* and antimalarial drugs but also show that the QSAR models for *T. gondii* cannot be reliably extended for screening antimalarial activity.
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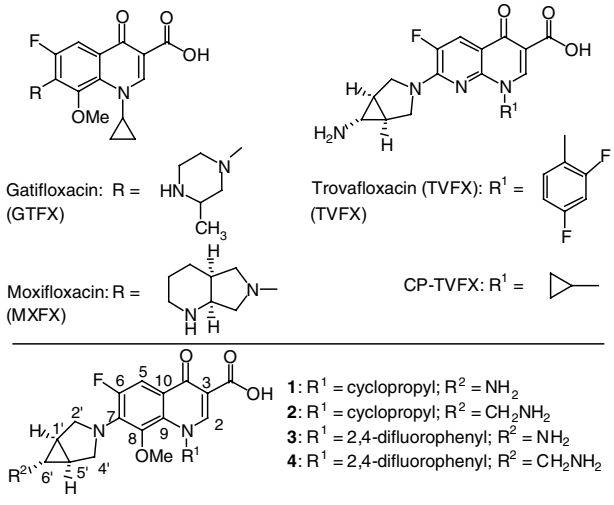
Fluoroquinolones have attracted much attention because of their broad spectrum of activity against various bacteria, mycobacteria, and parasites.[1–3] Indeed, the presence of a prokaryotic-like organelle in apicomplexan parasites, which comprise major human pathogens such as *Plasmodium* spp., *Toxoplasma gondii*, and other coccidia, may represent a unique target for antibiotics against these protozoa.[4–6] In this context and in order to discover new and potent antiparasitic fluoroquinolones derivatives, a QSAR analysis by molecular connectivity of a series of quinolones active against *T. gondii* was performed.[7] This analysis led us to identify their main active pharmacophores and to design new virtual structures that should display higher or at least comparable biological activities to those of already known fluoroquinolone drugs. Among the computer-designed fluoroquinolones, we selected the four fluoroquinolones **1**–**4** (Scheme 1) for which a high *anti-Toxoplasma* activity was predicted (see Table 1) and synthesis seemed feasible.



Scheme 1. Chemical structure of the target fluoroquinolones **1**–**4** and of known close analogs.

Keywords: Fluoroquinolone; Protozoa; QSAR; Malaria; Parasites.
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doi:10.1016/j.bmcl.2004.03.070

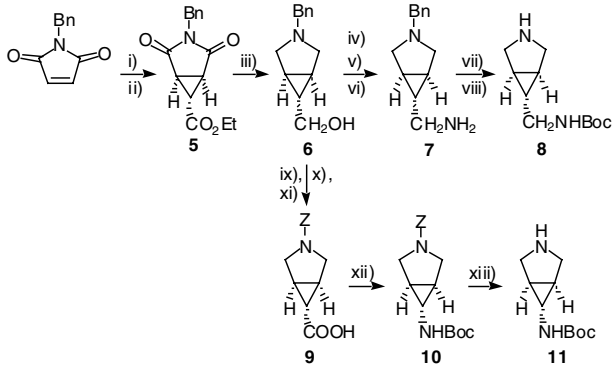
Table 1. Activities of fluoroquinolones against *Toxoplasma gondii* (RH) on MRC5 fibroblast tissue cultures,[7] against hepatic stages of *Plasmodium yoleii yoleii* (265 BY strain), and against blood stages of chloroquine-resistant strain of *Plasmodium falciparum* (NF54-R)[17]

Compound	*T. gondii* IC_{50} (μM) [predicted]	*P. yoleii yoleii* IC_{50} (μM) (±SD)	*P. falciparum*[f] IC_{50} (μM) (±SD)
1	2.1 [1.15][a] (1.6–3.1)[b]	73.1 (8.2)	92 (13)
2	66 [1.21][a] (54–87)[b]	Not active	100 (1)
3	32 [0.58][a] (26–42)[b]	Not active	105 (20)
4	37 [0.63][a] (30–48)[b]	Not active	40 (2)
TVFX	0.96 [1.2][c] 2.93[d]	74.0 (47.8)[e]	22 (12)[e]
CP-TVFX	1.68 [2.3][c]	—	—
GTFX	12.7 [33.8][c]	Not active[e]	29 (1)[e]
MXFX	10.9 [6.2][c]	Not active[e]	45 (18)[e]

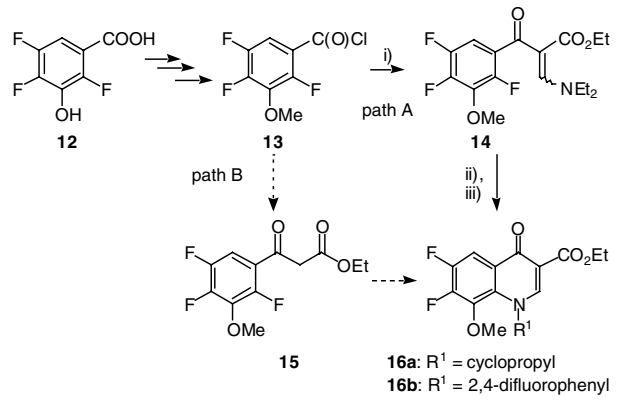
[a] The predicted value was calculated using the protocol described in Ref. 7.
[b] 95% Confidence interval.
[c] Data from Ref. 7.
[d] Data from Ref. 8.
[e] Data from Ref. 17.
[f] For comparison, the IC_{50} of chloroquine is of 0.57 ± 0.4 μM.[17]

It should be emphasized that the fluoroquinolones **1**–**4** contain the basic C8-methoxy quinoline structure of gatifloxacin (GTFX) and moxifloxacin (MXFX), and are also close analogs of trovafloxacin (TVFX) and cyclopropyl-TVFX[8] (Scheme 1), these four latter drugs being among the most *anti-Toxoplasma* active drugs known so far (see Table 1).[7–9]

We describe here the synthesis of these fluoroquinolones and also report on their in vitro activity against *T. gondii* and against blood and hepatic stages of *Plasmodium* spp. since these parasites might share common drug targets to quinolones.[5] The known amino-azabicyclohexane **11** and its methylamino analog **8** (Scheme 2), as well as the difluoroquinolone **16a**[10] and its new analog **16b** (Scheme 3) constitute the key synthons to the target



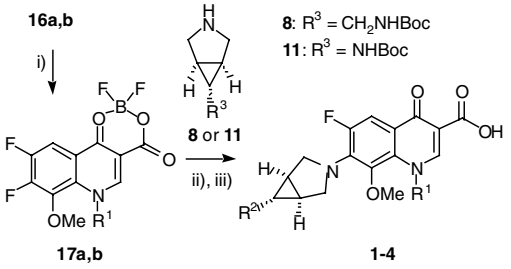
Scheme 2. Synthetic pathway to the *N*-Boc-protected amino-azabicyclohexanes **8** and **11**: (i) N_2CHCO_2Et, Et_2O, rt, 23 h; (ii) 185 °C, 2 h; (iii) $LiAlH_4$, THF, reflux, 48 h; (iv) $(ClCO)_2$, DMSO, NEt_3, CH_2Cl_2, −75 °C, 19 h, rt; (v) CH_3COONa, $HONH_2 \cdot HCl$, EtOH, rt, 16 h; (vi) $LiAlH_4$, THF, reflux, 15 h; (vii) $(Boc)_2O$, NEt_3, 9:1 dioxane/H_2O, rt, 4 h; (viii) $HCOONH_4$ or H_2, Pd/C, EtOH, rt, 1 h; (ix) H_2/Pd/C, MeOH, 0 °C, 23 h; (x) K_2CO_3, $ClC(O)OCH_2Ph$ (ZCl), 1:1 dioxane/H_2O, rt, 15 h; (xi) Jones reagent, acetone, 0 °C then rt, 2 h; (xii) *t*-BuOH, NEt_3, $(PhO)_2P(O)N_3$, reflux, 44 h; (xiii) H_2/Pd/C, MeOH, 0 °C, 5 h.



Scheme 3. Synthetic pathway to the difluoroquinolone **16a**,**b** synthons. Path A: (i) $Et_2NCH{=}CHCO_2Et$, NEt_3, toluene, 90 °C, 5 h; (ii) R^1NH_2, 1:2 EtOH/Et_2O, rt, 3 h; (iii) K_2CO_3, DMF, 100 °C, 5 h. Path B: (a) $[O_2CCHCO_2Et]^{2-}$ $2Li^+$, THF; (b) $(EtO)_3CH$, Ac_2O; (c) R^1NH_2, EtOH, (d) NaH, THF.[10]

and original fluoroquinolones **1**–**4** whose syntheses are shown in Scheme 4. The synthesis of the two heterobicyclic derivatives **8** and **11** (Scheme 2) was performed from commercial *N*-benzylmaleimide in eight steps (about 10% overall yield) according to published procedures.[11]

The synthetic approach we used for the two difluoroquinolones **16a**,**b** (path A) was adapted from the method developed by Cecchetti et al., for the synthesis of similar fluoroquinolones.[12–14] Its key steps lie in the transformation of **14** into **16a**,**b**. This method is easier to implement (milder conditions and three steps instead of four) and more efficient than the method briefly depicted in path B that has originally been applied for the *N*-cyclopropyl **16a** derivative.[10,15] Standard literature procedures applied to 3-hydroxy-2,4,5-trifluorobenzoic acid **12**[10] led to the formation of the acid chloride **13**. Its reaction with ethyl 3-(diethylamino)acrylate in the



Scheme 4. Synthetic pathway to the target fluoroquinolones **1**–**4**: (i) $BF_3 \cdot Et_2O$, THF, reflux; (ii) CH_3CN, 7 days, reflux; (iii) 1:1 CH_2Cl_2/TFA (for R^1 and R^2 see Scheme 1).

presence of NEt_3 gave **14**. Transaminolyse of **14** with cyclopropylamine or 2,4-difluoroaniline and successive cyclization with potassium carbonate in DMF gave the target esters **16a**,**b**, respectively.

The fluoroquinolones **1**–**4** were then obtained by coupling the difluoroquinolones **16a**,**b** with the amines **8** or **11**, as outlined in Scheme 4. This conjugation needed the activation of the C-7 position of the ethyl esters **16a**,**b**.[12] This was performed by converting them into their respective boron difluoride complexes **17a**,**b** using boron trifluoride etherate in refluxing THF. The successive but nevertheless time-consuming (7 days) nucleophilic displacement of the C-7 fluorine atom in **17a** (resp. **17b**) with 2.5–3 equiv of the selected heterobicyclic amine **11** or **8** followed by *N*-Boc deprotection using an excess of 1:1 TFA/CH_2Cl_2 gave the desired fluoroquinolones **1** or **2** (resp. **3** or **4**), as their TFA salts[16] with 27% or 40% (resp. 40 or 47%) overall yields from **16a**,**b**.

The four fluoroquinolones **1**–**4** were tested in vitro on MRC5 fibroblast tissue cultures inoculated with the virulent RH strain of *T. gondii*,[7] and against blood stages of the chloroquine-resistant NF54-R strain of *P. falciparum* (NF54-R) and liver stages of *P. yoelii yoelii* (265 BY strain) according to published procedures.[17] The results are collected in Table 1. These derivatives, predicted to be active by our molecular connectivity QSAR analysis against *T. gondii* were indeed found to be active, though only one of the four derivatives, that is, **1**, displayed a comparable inhibitory activity to that predicted. Moreover, **1** exhibited an *anti-T-gondii* IC_{50} value close to and higher than that of TVFX and of GTFX and MXFX, respectively, which are among the three most *anti-T-gondii* active fluoroquinolone drugs known. This derivative, like TVFX, was also active at a high concentration against the hepatic stages of *P. yoelii yoelii* (Table 1). Its inhibitory effect was also associated with alteration of schizont morphology and significant reduction of schizont sizes. While only **1** was active in this *Plasmodium* model, the four fluoroquinolones **1**–**4** were active against blood stages of the chloroquine-resistant strain of *P. falciparum,* though at much higher concentrations than that of chloroquine (Table 1). However, it is **4** of the four new fluoroquinolones which displays the highest antimalarial activity. This activity is close to that of TVFX, GTFX, and MXFX (Table 1). These data confirm the potential of quinolones as antimalarial drugs.

Our results show that the QSAR models developed for *T. gondii* cannot be reliably extended for screening antimalarial activity. Indeed, the *T. gondii* inhibitory activity decreased along the series TVFX ∼**1**>MXFX∼GTFX>**3**∼**4**>**2** while the *P. falciparum* inhibitory activity decreased along the series TVFX∼GTFX>**4**∼MXFX>**1**∼**2**∼**3**.

Concerning SAR for *anti-T-gondii* and *anti-Plasmodium* activity, our data show that combining the basic cyclopropyl–quinoline structure of GTFX or MXFX with the C-7 6-amino-3-azabicyclo[3.1.0]hexyl side chain of TVFX, as in **1**, increased substantially the activity against *T-gondii* (as well as the *anti-P. yoelii yoelii* activity of GTFX or MXFX which are not active) but decreased the *anti-P. falciparum* activity of GTFX and MXFX. By contrast, a significant *anti-T.* (as well as an *anti-P. falciparum*) activity decrease was observed when this basic cyclopropyl–quinoline structure was combined with the 6-methylamino-3-azabicyclo[3.1.0]hexyl side chain as in **2**. It thus appears that the contribution of the C7 substituent to *anti-T-gondii* activity decreases along the series: 6-amino-3-azabicyclohexane (as in **1**)>3′-methylpiperazine (as in GTFX) ∼ piperidinopyrrolidine (as in MXFX)>6-methylamino-3-azabicyclohexane (as in **2**). For *anti-P. falciparum* activity, the tendency of the C7 substituent contribution is as follow: 3′-methylpiperazine (as in GTFX)∼piperidinopyrrolidine (as in MXFX)>6-methylamino-3-azabicyclohexane (as in **2**)>6-amino-3-azabicyclohexane (as in **1**).

In line with literature,[18] our data confirm further that replacing the cyclopropyl in **1** by the 2,4-difluorophenyl as in **3** induces a marked *anti-T-gondii* and *anti-P. yoelii yoelii* activity decrease. This replacement has almost no incidence on *anti-P. falciparum* activity. When both the 6-methylamino-3-azabicyclo[3.1.0]hexyl side chain and the 2,4-difluorophenyl were combined with the basic quinoline structure GTFX or MXFX as in **4**, a more drastic *anti-T. gondii* activity decrease was observed while the *anti-P. falciparum* activity was almost not affected. Our data show further that replacing the basic naphthyridine structure of CP-TVFX by the basic quinoline one of **1**, (which is equivalent to replacing the intracyclic N atom by a C-methoxy group) had no effect on the *anti-T. gondii* activities.

Finally, our results show the usefulness and the limits of our predictive QSAR models for *T. gondii*. Indeed, the four computer-designed fluoroquinolones were active but only one out of these derivatives was as active as predicted, showing that the models remain of interest to orient synthesis of active *anti-T. gondii* quinolones. Concerning *Plasmodium* spp., further studies are in progress to define more adapted and specific QSAR models for the design of new antimalarial drugs.

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16. The chemical structure of compounds **1**–**4** was unambiguously established by ^{1}H, ^{13}C-DEPT, HSQC ^{1}H–^{13}C NMR, ESI-MS. Compound **1** (TFA salt): ESI-MS: $(M+H)^{+}$ 374.2, $(M+Na)^{+}$ 396.2 (in agreement with the mass calculated for $M = C_{19}H_{20}FN_3O_4$). For atom numbering, see Scheme 1: ^{1}H NMR (1:4 CD_3CN/D_2O): 0.93–1.13 (m, 4H, CH_2 cyclopropyl), 2.04 (s, 2H, $H_{1'}$ and $H_{5'}$), 2.54 (s, 1H, $H_{6'}$), 3.52 (s, 3H, OCH_3), 3.59, 3.82 (d, d, 2H, 2H, $H_{2'}/H_{4'}$ *exo*/*endo*, $^{2}J_{H-H} = 9.8$ Hz), 4.10 (m, 1H, CH, cyclopropyl), 7.63 (d, 1H, H_5,$^{3}J_{H-F} = 13.1$ Hz), 8.75 (s, 1H, H_2). ^{13}C NMR (DMSO-d_6): 9.3 (s, CH_2 cPr), 20.9 (s, $C_{1'}$ and $C_{5'}$), 29.9 (s, $C_{6'}$), 41.1 (s, CH cPr), 51.1/51.2 (2s, $C_{2'}$ and $C_{4'}$), 62.4 (s, OCH_3), 106.7 (d, C_5, $^{2}J_{C-F} = 24.1$ Hz), 106.7 (s, C_3), 117.4 (q, TFA, CF_3, $^{1}J_{C-F} = 299.0$ Hz), 119.9 (d, C_9, $^{4}J_{C-F} = 8.0$ Hz), 134.5 (s, C_{10}), 136.1 (d, C_7, $^{2}J_{C-F} = 11.4$ Hz), 144.8 (d, C_8, $^{2}J_{C-F} = 6.9$ Hz), 150.8 (s, C_2), 154.9 (d, C_6, $^{1}J_{C-F} = 250.1$ Hz), 158.8 (q, TFA, COOH, $^{2}J_{C-F} = 31.7$ Hz), 166.3 (s, C_4), 176.5 (s, COOH). ^{19}F NMR (1:4 CD_3CN/D_2O): −74.4 (6.3F, TFA), −117.4 (1F, F_6).
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