Supplementary Material (ESI) for Medicinal Chemistry Communications

Ester prodrugs of ciprofloxacin as DNA-gyrase inhibitors: synthesis, antiparasitic evaluation and docking studies

Faustine Dubar, René Wintjens, Érica S. Martins-Duarte, Rossiane C. Vommaro, Wanderley de Souza, Daniel Dive, Christine Pierrot, Bruno Pradines, Alexandre Wohlkonig, Jamal Khalife, and Christophe Biot*

ELECTRONIC SUPPLEMENTARY DATA

1. Chemistry
1.1. General materials and methods S2
1.2. Synthetic procedures S2
1.3. Table SI1. S5
2. In vitro biological assays
2.1. Blood-schizontocial activity against *Plasmodium falciparum* S6
2.2. *Anti-Toxoplasma* gondii activity S6
2.3. Cytotoxicity S7
3. Modeling studies
3.1. Amino acid sequence alignments S8
3.2. Models of *P. falciparum* and *T.gondii* DNA gyrase S9
3.3. Ligands preparation S10
3.4. Docking protocol S10
4.References S11

1. Chemistry

1.1. General materials and methods

Nuclear magnetic resonance (^{1}H, ^{13}C, and ^{19}F NMR) spectra were recorded at room temperature on a Brüker AC 300 spectrometer. TMS was used as an internal standard and $CDCl_3$ as the solvent. ^{1}H NMR analyses were obtained at 300 MHz (s: singlet, br s: broad singlet, d: doublet, t: triplet, dd: double doublet, m: multiplet); ^{13}C NMR analyses were obtained at 75.4 MHz; and ^{19}F NMR analyses were obtained at 282 MHz. The chemical shifts (δ) are given in parts per million relative to TMS (δ = 0.00). Mass spectra were recorded by means of a Waters Micromass Quattro II triple quadrupole LC mass spectrometer equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources. Melting points were determined on a Köfler apparatus and are uncorrected. Column chromatography, carried out on silica gel (Macherey-Nagel Kieselgel 60 M) was used for the purification of compounds. Reactions were monitored by thin-layer chromatography (TLC) using coated silica gel plates, detection by UV lamp. The purity degree of all compounds were checked by combustion analysis and proved to be higher than 95%.

1.2. Synthetic procedures

1.2.1. Synthesis of 2-adamanta-1ylethanol

A solution of 2-adamant-1-ylacetic acid (0.500 g, 0.0026 mol) in anhydrous THF (10 mL) was added dropwise to a suspension of $LiAlH_4$ (0.200 g, 0.0053 mol) in anhydrous THF (10 mL) under N_2 atmosphere. The mixture was stirred at room temperature for 12h. The mixture was then hydrolyzed by addition of ethanol (10 mL) and ice. The solution was acidified by 5% aq. citric acid (10 mL) and then extracted by diethyl ether (3×10 mL). The organic layers were washed twice with 5% aq. $NaHCO_3$ (5mL) and then with water (10 mL). The organic layer was dried over anhydrous $MgSO_4$. After removing the drying agent, the solvent was removed under reduce pressure. A white solid (0.458 g, 0.0025 mol) was obtained (98% yield).

δ_H ($CDCl_3$, 300 mHz) 3.71 (t, J=7.23 Hz; 2H, -CH_2-CH_2-OH); 1.94 (m; 3H, 3C*H*β-Ad); 1.65 (m; 12H, 3CH_2α-Ad and 3CH_2γ-Ad); 1.38 (t, J = 7,23 Hz; 2H, , -CH_2-CH_2-OH). δ_C ($CDCl_3$, 75.4 mHz) 59.2 (-CH_2-*C*H_2-OH); 47.5 (-*C*H_2-CH_2-OH); 43.1 (3*C*H_2α-Ad); 37.4 (3*C*H_2γ-Ad); 28.9 (3*C*Hβ-Ad).

1.2.2. Synthesis of 2-adamant-1-ylacetyle chloride

2-adamant-1-ylethanol (0.690 g, 3.8 mmol) was dissolved in pyridine (0.15 mL) and thionyle chloride (1 mL). The mixture was heated at 70°C for 2h. The mixture was cooled at room temperature then was hydrolyzed by water (10 mL). The aqueous layer was extracted with dichloromethane (3×10 mL). The organic layers were dried over anhydrous $MgSO_4$. After removing the drying agent, the solvent was removed over reduce pressure. A beige solid (0.530g, 2.7 mmol) was obtained (70 % yield).

δ_H ($CDCl_3$, 300 mHz) 3.52 (t, J=8.04 Hz; 2H, CH_2-CH_2-Cl); 1.93 (s; 2H, -CH_2-CH_2-Cl); 1.61 (m; 9H, 3C*H*β-Ad and 3CH_2α-Ad); 1.49 (m; 3CH_2γ-Ad). δ_C ($CDCl_3$, 75.4 mHz) 40.9 (-CH_2-*C*H_2-Cl); 42.3 (-*C*H_2-CH_2-Cl); 43.6 (3*C*H_2α-Ad); 37.5 (3*C*H_2γ-Ad); 29.1 (3*C*Hβ-Ad); 20.8 (C_{IV}-Ad).

1.2.3. Synthesis of 1-(2-(adamantan-1-yl)ethyl)piperazine

2-adamant-1-ylacetyle chloride (0.400 g, 2 mmol), piperazine (1.220 g, 14 mmol) and K_2CO_3 (1.180 g, 8.6 mmol) were dissolved in acetonitrile (10 mL). The mixture was refluxed for 24h. The mixture was cooled at room temperature then was hydrolyzed by water (20 mL). The mixture was extracted by dichloromethane (3×20 mL). The organic layers were dried over anhydrous $MgSO_4$. After removing the drying agent, the solvent was evaporated under reduce pressure. A beige solid (0.430 g, 1.7 mmol) was obtained (87% yield).

δ_H ($CDCl_3$, 300 mHz) 2.83 (t, J=8.81 Hz; 4H, 2C*H*$_2$ of piperazine); 2.37 (m; H, -CH_2-C*H*$_2$-piperazine); 2.25 (t, J=9.12 Hz; 4H, 2C*H*$_2$ of piperazine); 1.86 (m; 3H, 3C*H*-Ad); 1.59 (m; 6H, 3C*H*$_2$-αAd); 1.42 (m; 6H, 3C*H*$_2$-γAd); 1.20 (m, 2H, -C*H*$_2$-CH_2-piperazine). δ_C ($CDCl_3$, 75.4 mHz) 54.6 (-CH_2-*C*H_2-piperazine); 52.8 (*C*H_2 of piperazine); 46.0 (*C*H_2 of piperazine); 42.2 (3*C*H_2-γAd); 40.7 (-*C*H_2-CH_2-piperazine); 37.2 (3*C*H_2-αAd); 28.5 (3*C*H-Ad); 21.7 (C_{IV}-Ad).

1.2.4. Synthesis of ethyl 7-(4-(2-((adamantan-1-yl)ethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (5)

1-(2-(adamantan-1-yl)ethyl)piperazine (0.186 g, 0.75 mmol) and ethyl 1-cyclopropyl-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **10** (0,110 g, 0.37 mmol) were dissolved in acetonitrile (6 mL). The mixture was refluxed for one week. The mixture was cooled at room temperature then was hydrolysed by water (20 mL). The mixture was extracted by dichloromethane (3×20 mL). The organic layers were dried over anhydrous $MgSO_4$. After removing the drying agent, the solvents were evaporated under reduce pressure. The crude product was purified by column chromatography over silica gel using AcOEt/Petroleum (7:3) then AcOEt/Petroleum ether/MeOH (9:0.5:0.5). A beige solid (0.117 g, 0.22 mmol) was obtained (30 % yield).

δ_H ($CDCl_3$, 300 mHz) 8.44 (s; 1H, H_2); 7.95 (d, J=13.62 Hz; 1H, H_5); 7.20 (s; 1H, H_8); 4.31 (q, J=7.08 Hz; 2H, CH_3-C*H*$_2$-O); 3.36 (quin, *J* = 3.27 Hz; 1H, C*H* (cyclopropyle)); 3.22 (t, J=4,05 Hz; 4H, 2C*H*$_2$ of piperazine); 2.60 (t, J=4.29 Hz; 4H, 2C*H*$_2$ of piperazine); 2.37 (t, J=7,83 Hz; 2H, -CH_2-C*H*$_2$-piperazine); 1.89 (m; 3H, 3C*H*-Ad); 1.64 (m; 6H, 3CH_2γ-Ad); 1.46 (m; 6H, 3C*H*$_2$α-Ad); 1.33 (t, J=7.08 Hz; 3H, C*H*$_3$-CH_2-O); 1.24 (m; 4H, C*H*$_2$ (cyclopropyle) and -C*H*$_2$-CH_2-piperazine); 1.06 (m; 2H, C*H*$_2$ (cyclopropyle)). δ_C ($CDCl_3$, 75.4 mHz) 173.1 (-*C*=O); 166.0 (-*C*OO); 153.2 (*C*$_6$); 148.2 (*C*$_2$); 144.6 (*C*$_7$); 138.0 (*C*$_{10}$); 122.7 (*C*$_9$); 113.3 (*C*$_5$); 110.4 (*C*$_3$); 104.7 (*C*$_8$); 60.9 (CH_3-*C*H_2-O); 53.2 (2*C*H_2 of piperazine); 52.8 (CH_2-*C*H_2-piperazine); 50.0 (2*C*H_2 of piperazine); 42.6 (3*C*H_2α-Ad); 41.0 (-*C*H_2-CH_2-piperazine); 37.1 (3*C*H_2γ-Ad); 34.5 (*C*H (cyclopropyle)); 31.8 (C^{IV}-Ad); 28.6 (3*C*H-Ad); 14.4 (-*C*H_3-CH_2-O); 8.1 (*C*H_2 (cyclopropyle)). δ_F ($CDCl_3$, 282 mHz) -123,5 (F). MS (EI): m/z 522 [MH^+]. Anal. (C, H, N).

1.2.5. Synthesis of 7-(4-Benzyl-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester

A solution of ethyl 1-cyclopropyl-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **10** (0.071 g, 0.24 mmol), 1-benzylpiperazine (0.142 g, 0.81 mmol) and triethylamine (110µL,

0.75 mmol) in CH_3CN (4mL) was refluxed for one week, under N_2. After cooling at room temperature, the mixture was hydrolysed then extracted with CH_2Cl_2 (3×20 mL). The organic layers were dried over $MgSO_4$, filtered and evaporated. The yellow oily residue was purified by flash chromatography on silica gel (99.5:0.5 to 95:5 CH_2Cl_2/MeOH) affording the product (0.067 g, 0.15 mmol) 449.22 as a brown-orange solid (64%).

δ_H ($CDCl_3$, 300 mHz) 1.10 (m, 2H), 1.27 (m, 2H), 1.37 (t, *J* = 7.2 Hz, 3H), 2.66 (m, 4H), 3.26 (m, 4H), 3.41 (t, *J* = 7.2 and 3.6 Hz, 1H), 3.59 (s, 2H), 4.34 (q, *J* = 7.0 Hz, 2H), 7.21 (d, *J* = 8.1 Hz, 1H), 7.30 (m, 5H), 7.88 (d, *J* = 13.8 Hz, 1H), 8.42 (s, 1H); δ_C ($CDCl_3$, 75.4 mHz) 8.1, 14.5, 34.5, 48.5, 50.0, 52.8, 60.7, 63, 104.8 (d, *J* = 3 Hz), 110.0, 112.9 (d, *J* = 23 Hz), 122.7 (d, *J* = 7 Hz), 127.2, 128.3, 129.2, 137.8, 137.9 (d, *J* = 1 Hz), 144.5 (d, *J* = 11 Hz), 148.0, 153.8 (d, *J* = 249 Hz), 165.8, 173.0 (d, *J* = 2.3 Hz); δ_F ($CDCl_3$, 282 mHz) -123,5 (dd, *J* = 13.4 and 7.3 Hz); MS (EI) : m/z 450 [MH^+]. Anal. (C, H, N).

Stability Studies in Aqueous Solutions. The rates of chemical hydrolyses of ester prodrugs **2**−**5**, were studied at 37 °C in aqueous phosphate buffer of pH 7.4 (150 mM NaCl, 4 mM NaH_2PO_4, 25 mM $NaHCO_3$). A 1 mM stock solution of ester prodrugs **2**−**5** in 10% aqueous DMSO was diluted in preheated buffer, and the 100 μM solutions of ester prodrugs **2**−**5** were placed in a thermostatically controlled water bath at 37 °C. The 100 μL samples of the solutions were collected at different times (*t* = 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 20, 24, 30, 48 and 76 h). The 100 μL samples were added to 100 μL of EtOH and then were directly injected through a 50 μL loop for HPLC analysis. Analytical HPLC was performed on a Shimadzu system equipped with a C18 Bio-Rad Bio-Sil C18 A/B 90-5 column (4.6 mm × 250 mm) and a UV detector set at 278 nm. The following solvent systems were used: (A), 0.05% trifluoroacetic acid (TFA) in H_2O and (B) 0.05% TFA, 20% H_2O, 80% CH_3CN, with the following conditions: (A) 1 min, a linear gradient from (A) to (B), 0−100% for 30 min, (A) 5 min. HPLC retention times (HPLC t_R) were obtained at flow rates of 1 mL/min. In the presence of human serum, t_R = 15.47 min for CIPRO **1**, t_R = 17.53 min for **2**, t_R = 24.04 min for **3**, t_R = 17.63 min for **4**, and t_R = 26.42 min for **5**.

Stability Studies in RPMI−10% Human Serum. The rates of enzymatic hydrolysis of ester prodrugs **2-5** were studied in RPMI−10% human serum at 37 °C. The reactions were initiated by dissolving a 1 mM stock solution of ester prodrugs **2-5** in 10% aqueous DMSO in the preheated medium, and the 100 μM solutions of ester prodrugs **2-5** were placed in a thermostatically controlled water bath at 37°C. The 100 μL samples of the solutions were collected at different times (*t* = 0, 0.5, 1, 2, 3, 4, 20, 24, 30, 45, 53, 70 and 76 h). The 100 μL samples were added to 100 μL of EtOH, then were centrifuged for 1 min at 8000 rpm. The supernatant was directly injected through a 50 μL loop for HPLC analysis. The following solvent systems were used: (A), 0.05% trifluoroacetic acid (TFA) in H_2O and (B) 0.05% TFA, 20% H_2O, 80% CH_3CN, with the following conditions: (A) 5 min, a linear gradient from (A) to (B), 0−100% for 30 min, (A) 5 min. HPLC retention times (HPLC t_R) were obtained at flow rates of 1 mL/min. In the presence of human serum, t_R = 15.47 min for

CIPRO **1**, t_R = 17.53 min for **2**, t_R = 24.04 min for **3**, t_R = 17.63 min for **4**, and t_R = 26.42 min for **5**.

Table SI1. Predicted log*P* and pK_a values of the organic derivatives

Compound	log*P*	pK_a
2	2.29	8.67
monoprotonated **2**	-0.93	8.67
1, zwiterionic form	-4.20	8.68 and 5.76
1, neutral form	1.71	8.68 and 5.76
4	4.35	6.69
monoprotonated **4**	0.95	6.69
7, zwiterionic form	-2.32	6.74 and 5.70
7, neutral form	3.77	6.74 and 5.70
5	5.25	7.58
monoprotonated **5**	1.85	7.58
8, zwiterionic form	-1.42	7.58 and 5.74
8, neutral form	4.66	7.58 and 5.74

Values of log*P* and pK_a were calculated using Marvin, Calculator Plugin from ChemAxon 5.2[1]

2. In vitro biological assays

2.1. Blood-schizontocial activity against *Plasmodium falciparum*

The antimalarial activity of all compounds was tested against *P. falciparum* strains 3D7 (Africa), W2 (Indochina), and Tm90C2b (Thailand).Parasites were maintained in culture in RPMI 1640 (Invitrogen, Paisley, United Kingdom) supplemented with 10% human serum (Abcys S.A., Paris, France) and buffered with 25 mM HEPES and 25 mM $NaHCO_3$. Parasites were grown in A-positive human serum (Etablissement Français du Sang, France) under a controlled atmosphere (10 % O_2. 5 % CO_2 and 85 % N_2 at 37 °C, humidity 95 %). All compounds were dissolved in DMSO 1% (v/v) in RPMI. Two fold serial dilutions with final concentrations ranging from 0.005 µM to 100µM were prepared in DMSO 1% in RPMI for fluoroquinolones (and from 0.15 to 500 µM for ciprofloxacin) and distributed into Falcon 96-well plates just before use.

For in vitro isotopic microtests 25 µL/well of the molecules and 200 µL/well of the suspension of parasitized erythrocytes (final parasitemia 0.5 % for the 48h-test and 0.2% for the 96h-test, > 95% of young trophozoites; final hematocrit 1.5%) were distributed in 96-well plates. Parasite growth was assessed by adding 1 µCi of [^{3}H]hypoxanthine with a specific activity of 14.1 Ci/mmol (Perkin-Elmer, Courtaboeuf, France) to each well at 0h for the 48h exposure test and at 48h for the 96h exposure test. The plates were then incubated for 48 h (or 96h) in the controlled atmosphere previously described. Immediately after incubation plates were frozen and then thawed to lyse erythrocytes. The content of each well was collected on filter microplates (Unifilter GF/B; Perkin-Elmer) and washed by using a cell harvester (Filter-Mate Cell Harvester; Perkin-Elmer). Filter microplates were dried, and 25 µL of scintillation cocktail (Microscint O; Perkin-Elmer) was placed in each well. Radioactivity incorporated by the parasites was measured with a scintillation counter (Top Count; Perkin-Elmer).

The IC_{50} (the drug concentration leading to 50 % of the uptake of [^{3}H]hypoxanthine by parasites in drug-free control wells) was determined by nonlinear regression analysis of log-dose/response curves.

2.2. *Anti-Toxoplasma* gondii activity

Parasites and cells: Tachyzoites from the virulent RH strain of *T. gondii*, isolated from human brain[2], were used for the in vitro experiments and were maintained by intraperitoneal (i.p.) passages in Swiss mice. For the in vitro anti-proliferative assays approximately 5×105 cells were placed in a 24-well tissue culture plate 24h before the experiment. The cells were infected with freshly obtained parasites, resuspended in RPMI without fetal bovine serum (FBS) at a ratio of 10:1 parasite/host cell. Tachyzoites were allowed to interact for 1h and then the cell monolayers were washed twice with phosphate-buffered saline (PBS) to remove non-adherent extracellular parasites. Different concentrations of different fluoroquinolones were added to the infected cells after 6h of infection and incubated for 24 or 48h at 37°C (assays were performed in triplicate). After treatment, samples were fixed with Bouin, stained with Eosin & Hematoxiline and observed in a light microscope. The parasite proliferation index was determined by the examination of at least 400 cells from two different coverslips

and was determined by multiplying the percentage of infected cells by the total number of intracellular parasites per total number of cells[3].

For IC_{50} (concentration for 50% growth inhibition) calculations, the percentage of growth inhibition was plotted as a function of the drug concentration by fitting the values to the non-linear curve analysis. The regression analyses were performed using Sigma Plot 8.0 software (Systat Software Inc, Chicago, IL, USA).

2.3. Cytotoxicity

LLC-MK2 cell cultures (kidney, Rhesus monkey, Macaca mulata – ATCC CCL7, Rockville, MD/USA) were maintained in RPMI medium with 5% Foetal Bovine Serum (FBS) at 37°C in an atmosphere of 5% CO_2. The effect of the drugs on LLC-MK2 cells was evaluated by MTS assay, which is based on the reduction of the salt MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) into soluble formazan by mitochondrial dehydrogenase enzymes in metabolically active and viable cells. For that, 2x105 cells were placed in a 96-well tissue plate and treated with different concentrations of compounds (5 to 30µM) for 48h. At the end of incubation time, cells were washed with PBS and each well was filled with 100µL of PBS + 10mM Glucose and 20µL of MTS reagent (Promega, Madison WI, USA) added. The absorbance was read at 490nm after 3h of incubation and cytotoxicity was calculated as the percentage of viable cells versus untreated control cells.

The cytotoxic effect of compounds was also assessed using murine splenocytes stimulated by concanavalin A.[4] Cells isolated from BALB/c mice and washed twice in RPMI 1640 medium, were resuspended in RPMI 1640 supplemented with 0.1 mM non essential aminoacids (Cambrex Biosciences, Walkersville, USA), 4mM glutamine (Cambrex), 10% FBS, 5 µg/ml gentamycin, 50 µM β-mercaptoethanol (Merck), and 1 µg/ml concanavalin A (Sigma). Cells (2.10^5 cells /well in 100µl) were then seeded into 96-well flat-bottom tissue culture plates containing drug solutions (100 µl) serially diluted with complete culture medium. The plates were incubated for 72 h in a humidified atmosphere at 37 °C and 5% CO_2. Twenty µl of a stock solution of resazurin (alamar Blue, AbD Serotec, Oxford UK) were then added per well (final concentration 10 µM), and the plates were further incubated at 37°C for 24 h. Optical densities were measured in a DYNEX MRX II plate reader with excitation wavelength at 570 nm and emission wavelength at 620 nm. The calculations (difference in reduction between control and treated cells) were done according to the recommendations of manufacturer.[5] IC50 values were determined as previously described.[6]

3. Modeling studies

3.1. Amino acid sequence alignments

The *P. falciparum* and *T. gondii* DNA gyrase sequences were obtained from Tr-EMBL data base. The NCBI-Blast server on EBI (http://www.ebi.ac.uk/Tools/sss/ncbiblast/) was used to search the closest sequence of topoisomerases in the RCSB Protein Data Bank. As we would like to model a ternary complex structures between ciprofloxacin and DNA gyrase, we chose the X-ray structure of a same complex but with the gyrase from *Staphylococcus aureus*. The high degree of sequence identity between the target DNA gyrases and *S. aureus* topoisomerase II (around 31%) indicates that this structure (PDB code: 2XCT) is a correct model, usable as template. The sequence alignments of *P. falciparum* and *T. gondii* DNA gyrases A and B with the corresponding proteins from *S. aureus* were performed using ClustalW[7], and then refined by hand.

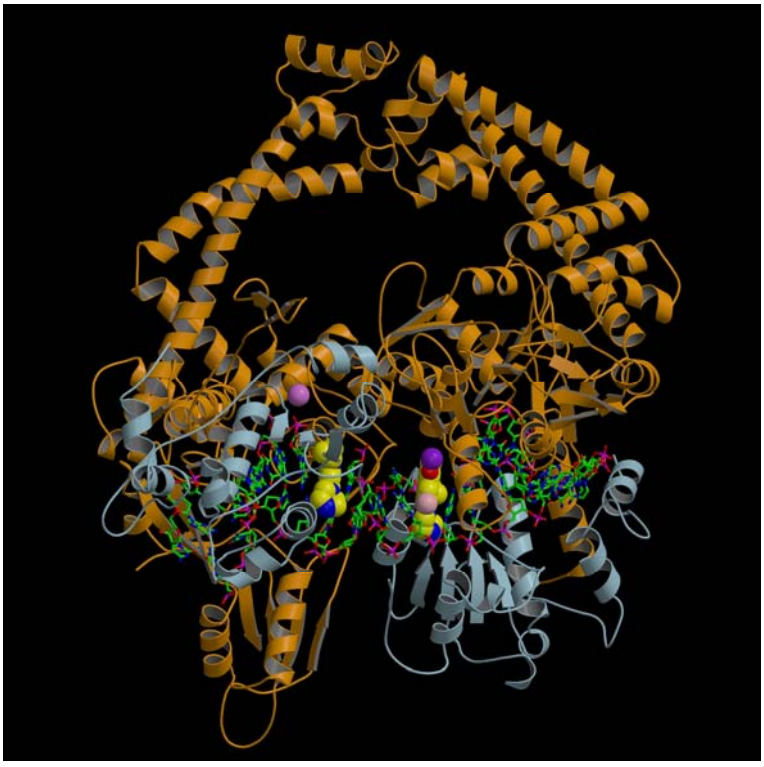
```
GyrA                10        20        30        40        50        60        70        80
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Q99XG5     NERNITSEMRESFLDYAMSVIVARALPDVRDGLKPVHRRILYGLNEQGMTPDK-SYKKSARIVGDVMGKYHPHGDSSIYE
Q8I0X3     YDVEICEILSKSFLSYANFLILNRCLCDYRDGLKTVQRRIIWSMYEINKGIDKKGYKKCARIVGEVIGKYHPHGDKSVYD
B9PYK0     LDTQFVEELEKSFLCYAYSTILSRALPDVRDGLKPVHRRLIYAMQQLHLHPSG-SFRKCARVVGEVLGKFHPHGDQAVYD
                                                                                  #   #
                    90        100       110       120       130       140       150       160
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Q99XG5     AMVRMAQDFSYRYPLVDGQGNFGSMDGDGAAAMRYTEARMTKITLELLR-DINKDTIDFIDNYDGNEREPSVLPARFPNL
Q8I0X3     ALVRLAQKHHNNNLLIKGYGNFGSVEYN-AAAMRYTEAKISSFCYDILLDEINDENVEYIKNFDGNEREPKVLCSKIPLL
B9PYK0     ALVRLAQTFVARHPLIEGHGNFGSVDGDPPAAMRYTECRLTRFCEDALLKNLDDKVVPFRPNFDANEREPVVFPASVPLV
                                           *
                    170       180       190       200       210       220       230       240
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Q99XG5     LANGASGIAVGMATNIPPHNLTELINGVLSLSKNPDISIAELMEDIEGPDFPTAGLILGKSGIRR-AYETGRGSIQMRSR
Q8I0X3     LINGCSGIAVSILSSIPCHNLIDVANCCINFLINENIRDDELFHIIKGPDFSTGGIIISKYDILKNIYNSGKGNFEIRSN
B9PYK0     LIQGSSGIAVGMSTQIPPHNLHEILDATIALIRDPELPDAELLRLVPGPDFPTGGLLVNSEEIPG-AYKEGRGRVLLRGR

                    250       260       270       280       290       300       310       320
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Q99XG5     AVIEERGG----------------------------GRQRIVVTEIPFQVNKARMIEKIAELVRDKK--IDGITDLRD
Q8I0X3     VFFEYIKNDKKVITKHIN--------DLSSIENSDIDKLTKKIIIKNLPPNVKPNELIENIINLLNDKKNEHDNILLRIR
B9PYK0     FHLEGEDDDGDTVPGRDRNAEDEAERDEADRHVRNGRKNERRLVITELPYGVNKSTVMQVIAQMISVKF--LEGVTSVRD

                    330       340       350       360       370       380       390       400
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Q99XG5     ETSLRTGVRVVIDVRKD---ANASVILNNLYKQTPLQTSFGVNMIALVNG-RPKLINLKEALVHYLEHQKTVVRRRTQYN
Q8I0X3     DESEKEDMRIVLELKKHSQIEQIHNFLSYLFKYTNMQISYHCNFVCIGYENTYTQFSLKSFIKLWCNNRIKFIKTNYEIK
B9PYK0     ESDWR-GIRVVLVLRRD---ADAHTILTLLLKHTNLQVYIPMHLIALEKGTKPVRFSLKSMLLSWIAFRFHTLRRLLAAE

                    410       420       430       440       450       460       470       480
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Q99XG5     LRKAKDRAHILEGLRIALDHIDEIISTIRESDTDKVAMESLQQR-FKLSEKQAQAILDMRLRRLTGLERDKIEAEYNELL
Q8I0X3     NKNLQKQLNIIDLYLIIQNKILDIITFFQKNQNIEQIQLYLKNN-FKLNPEQIKYILSIKLQKLINIKNIDFISQRNKIM
B9PYK0     EAERKARSHLLEGLLRAVSLMDDVVKTVRESLSADDAKQKLTEPPLGFSPAQAQALLRLTLSRLTRLERRQLEEEAKAQK

                    490       500       510       520
           ....|....|....|....|....|....|....|..
Q99XG5     NYISELETILADEEVLLQLVRDELTEIRDRFGDDRRTEIQLG
Q8I0X3     HQIKLNDEIINNVQNIKNLIIQELIYIKNKYGIHNLNKQCII
B9PYK0     DRLSELASLLAHDREIYELMVEELQAHKTRHKAPRKTKVLGL

GyrB-Toprim         10        20        30        40        50        60        70        80
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P66937     LPGKLADCSSKSPEE---CEIFLVEGDSAGGSTKSGRDSRTQAILPLRGKILNVEKAR-LDRILNNNEIRQMITAFGTGI
Q8I528     LPGKLVDCISDDISR---NEIFIVEGDSAAGSAKQARNREIQAILPLKGKILNVEKIKNNKRIFENSELKSLITALGLNV
B9Q192     LPGKLSDCSPTNARERLSKELFIVEGESAAGSAKQARDRRTQAILPLRGKILNVEKLGNFARIFENEELKALVAALGISV
                                  *
                    90        100       110       120       130       140       150       160
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P66937     GGDF----------------------DLAK-----------------------ARYHKIVIMTDADVDGAHIRTLLLTF
Q8I528     NYDNKNLKNNNILSNNKKGKNSKKKSDLKNSRFESTQNNNNILNKKKDILVDNTLRYGKVIIMTDADVDGEHIRILLLTF
B9Q192     TKAG-----------------EVSADLDG-----------------------LRYSRVIILTDADVDGAHIRSLLLTF
                                                                  * *
```

```
                170       180       190       200       210       220       230       240
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P66937    FYRFMRPLIEAGYVYIAQPPLYKLTQG-KQKYYVYNDRELDKLKSELNPT----------------------------
Q8I528    LYRFQKEIIENGNVYVACPPLYKITYN-----KFFDNSIKDIVTKQFNVTT----------------------------
B9Q192    FFRLQPELFRQGRIFVACPPLFKIAHFP-VPHKLLQDATAAFRKRPCSRATSAVPDEARAVHDAHTDEEERGDESDDEAG

                250       260       270       280       290       300       310       320
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P66937    ------PKWSIARYKGLGEMNADQLWETTMNPEHR------ALLQVKLED---AIEADQTFEMLMGDVV----------
Q8I528    ----KQSKFIIHTYSDQELNNLLKLLDKDKIASEQ------KNMQNKIKNKNMSSNGEPLSEYNEQSKTN------DIFV
B9Q192    RGGGKRLATETYVWSDEEVKRVFEVLRMHRESRKKEQRGPGKRMRGVAADAVLSGGEKPEHTAREPDRDDGRDGERGGEQ

                330
          ....|....|....|.
P66937    ---ENRRQFIEDNAVY
Q8I528    NEEGSTDSFIDDNVLF
B9Q192    DQEEGEERRSSDKTVP
```

Multiple sequence alignment between DNA gyrases sequences from *Staphylococcus aureus* (accession numbers Q99XG5 and P66937), *Plasmodium falciparum* (Q8I0X3 and Q8I528) and *Toxoplasma gondii* (B9PYK0 and B9Q192). The first alignment is for the gyrA subunit and the latter is for the Toprim domain of gyrB. The four 'catalytic' residues are indicated by (*) underneath sequences. The two key residues of the QRDR region, Ser84 and Asp84, are showed by (#) underneath sequences.

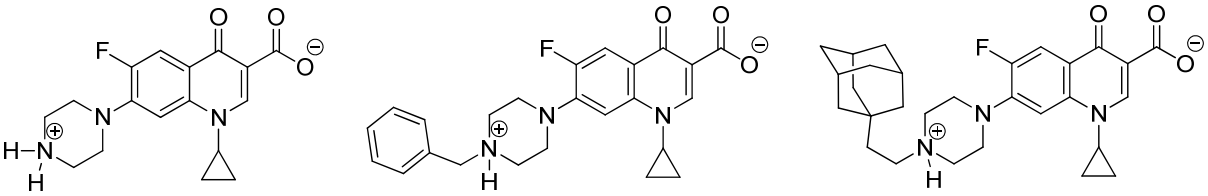
3.2. Models of *P. falciparum* and *T. gondii* DNA gyrases

Proteins reconstruction were achieved with Modeller9.8[8] on the entire sequence. All molecules present in the PDB file 2XCT, as the differents DNA strands, the ligands ciprofloxacin, the magnesium ions, as well as the four protein chains, were modeled.
The final models were chosen from according the best Modeller energy score.



Ribbon representation of the PfGyrase model. The gyrase A is coloured in orange and the gyrase B in light blue. The DNA is depicted with carbon atoms in green. The ciprofloxacin molecules are displayed with their carbon atoms in yellow. Mg ions bound to ciprofloxacin are in purple whereas catalytic Mg ions are in violet. The colouring sheme is oxygen in red, nitrogen in blue, fluor in pink, and phosphate in magenta. For clarity, unmodelled loops are not included.

3.3. Ligands preparation



Ciprofloxacin (**1**) (**7**) (**8**)

Ciprofloxacin and its derivatives can each exist as three chemical species (cationic, zwitterionic, and anionic) depending on the pH of the aqueous solution. For CIPRO, the experimentally measured pK_{a1} and p*K*a2 values are 5.9 and 8.2[9]. For **7**, the predicted pK_{a1} and p*K*a2 values are 5.7 and 6.7. For **8**, the predicted pK_{a1} and p*K*a2 values are 5.7 and 7.6. At physiological pH the major contribution is from the zwitterionic state. We therefore used the zwitterionic states of CIPRO and its derivatives in docking calculations.
The 3D structures were built and energy minimized using MOPAC2009.[10]

3.4. Docking protocol

Compounds **1**, **7** and **8** were docked into the QRDR of the homology-modeled DNA gyrases using AutoDock4.2.[11]. The graphical interface AutoDock Tools was used. Flexible torsion angles in the ligands were assigned with Autotors. The grid maps for each atom type found in the ligand structures were calculated using the auxilary program AutoGrid. The grid size was set to 30×30×48 points with 0.375 Å spacing, and the grid box was centered on the QRDR. The Lamarckian genetic algorithm was selected for ligand conformational searching.

4. References

1. http://www.chemaxon.com
2. Sabin, A. B., *Journal of American Medical Association*, 1941, **116**, 801.
3. de Araújo Jorge, T. C., and de Souza, W., *acta tropica*, 1984, **41**, 17.
4. Kelly, J. X., Smilkstein, M. J., Brun, R., Wittlin, S., Cooper, R. A., Lane, K. D., Janowsky, A., Johnson, R. A., Dodean, R. A., Winter, R., Hinrichs, D. J., and Riscoe, M. K., *Nature*, 2009, **459**, 270.
5. BUF012A/B alamarBlue™ Product datasheet. AbD Serotec,
6. Delhaes, L., Biot, C., Berry, L., Delcourt, P., Maciejewski, L. A., Camus, D., Brocard, J. S., and Dive, D., *Chembiochem*, 2002, **3**, 418.
7. Thompson, J. D., Higgins, D. G., and Gibson, T. J., *Nucleic Acids Res*, 1994, **22**, 4673.
8. John, B., and Sali, A., *Nucleic Acids Res*, 2003, **31**, 3982.
9. Barron, D., Jimenez-Lozano, E., and Barbosa, J., *J. Chromatogr. A*, 2001, **919**, 395.
10. MOPAC2009, J. J. P. S., Stewart Computational Chemistry, Colorado Springs, CO, USA,
11. Morris, G. M., Huey, R., and Olson, A. J., *Curr Protoc Bioinformatics*, 2008, **Chapter 8**, 8.