A novel predictive model for the anti-bacterial, anti-malarial and hERG cardiac QT prolongation properties of fluoroquinolones

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Abbreviations

FQs Fluoroquinolones, BBB Blood brain barrier, TdP Torsades de Pointes, GI gastrointestinal tract, IC_{50} Half maximal Inhibitory concentration , MIC Minimum inhibitory concentrations, ZW zwitterion, steady state uptake (R_{ss}), F blood plasma concentrations, CBP Chronic bacterial prostatis, BSA bovine serum albumin, MRP2 Multidrug resistance-associated protein 2 or specific organic anion transporter 1 (cMOAT) or ATP-binding cassette sub-family C member 2 (ABCC2), OATP organic anion uptake transporter, OCT organic cation influx transporter, $\Delta G_{desolvation}$ free energy of water desolvation, $\Delta G_{lipophilicity}$ free energy of lipophilicity or hydrophobicity, $\Delta G_{desolv,CDS}$ free energy of water desolvation of the cavitation dispersion solvent structure (CDS), $\Delta G_{lipo,CDS}$ free energy of lipophilicity or hydrophobicity for the CDS, DM dipole moment DM, SASA solvent accessible surface area, R^2 multiple correlation coefficient, F the F test of significance, SEE standards errors for the estimates, SE($\Delta G_{desolvation}$) standard errors of $\Delta G_{desolvation}$, SE($\Delta G_{lipophilicity}$), standard errors of $\Delta G_{lipophilicity}$, SE(Dipole Moment) standard errors for dipole moments, SE (Molecular Volume) standard errors for molecular volumes as calculated from "t" distribution statistics, QM quantum mechanics,

Introduction

We have previously shown[1-5] that four parameter equation (the general equation) can be successfully applied to:

- the transport of drugs and physiologically important molecules across the blood brain barrier (BBB): passive (simple permeation and facilitated) diffusion
- the competitive binding of statins to HMG CoA reductase
- the passive and active competitive transport of statins by organic anion transporters
- the competitive binding of a number of tyrosine kinase inhibitors and multi-kinase inhibitors to a range of kinase enzymes
- the active competitive transport of these tyrosine kinase inhibitors by the hOCT3, OATP1A2 and OCT1 transporters
- the binding of inhibitors to cyclin-dependent kinases (CDK) including multi-drug resistant CDK
- the binding of inhibitors to HIV-1 proteases and poly(ADP-ribose) polymerases (PARP)
- kinetic and equilibrium solubility in water of a large number of drugs
- intestinal drug absorption of a large number of drugs: permeation and active transport
- human intestinal permeation rates and human intestinal absorption for a large number of common drugs
- Caco-2, PAMPA, Everted Gut Sac, Sartorius membrane permeability of a large number of drugs

- drug-MRP2 permeability interactions in Caco-2 cells have been evaluated by determining the A-to-B and B-to-A permeability of MRP2 inhibitors

The model comprises four main drug properties calculated by quantum mechanical methods: (a) desolvation energy in water (b) lipophilicity or hydrophobicity based on the solvation energy in a hydrophobic solvent such as n-octane or n-octanol; lipophilicity being a measure of how well a drug can interact with lipophilic cell membrane bilayers, and hydrophobicity being a measure of non-polar interaction between a drug and the hydrophobic sectors of a protein (c) dipole moment in water, as a measure of the polar attraction between the drug and its receptor target or cell membrane (d) the molecular volume of the drug in water as a measure of how well the prospective drug fits into the cavity of the target receptor protein or active protein transporter, or how well a drug can diffuse through a cell membrane.

General Equation:

Transport or Binding = $\Delta G_{desolvation}$ + $\Delta G_{lipophilicity}$ + Dipole Moment + Molecular Volume

Or

Transport or Binding = $\Delta G_{desolv,CDS}$ + $\Delta G_{lipophilicity}$ + Dipole Moment + Molecular Volume

$\Delta G_{desolvation} = \Delta G_{electrostatic} + \Delta G_{CDS}$ where CDS is the cavity dispersion solvent structure. The CDS involves non-bulk solvent electrostatic contributions to the free energy of hydration. The SMD solvation model is based on $\Delta G_S^o = \Delta G_{ENP} + G_{CDS}$ where ENP is the electronic nuclear polarization: the change in the solute free energy due to electrostatic interactions between the solute and the bulk solvent and distortion of the solute's electronic structure in solution. The solvent is modelled as a dielectric continuum. The CDS represents first solvation shell effects. It involves atomic surface tension (geometry dependent proportionality constants). The CDS has been parameterized using extensive experimental data sets for optimization, and has the advantage of including a realistic experimentally based hydrogen bonding model. The CDS covers *shorter-range* polarization effects and shorter-range non-electrostatic effects such as cavitation, dispersion, and solvent structural effects (which includes both hydrogen bonding) and exchange repulsion effects.[6] [Marenich 2009[6]] $\Delta G_{desol,CDS}$ can be substituted for $\Delta G_{desolvation}$ where this non-polar term may be more representative of the interaction processes being studied, as for example in a ligand-protein binding study within the protein binding pocket where the dielectric constant for that environment is ca. ε=7-10 compared to ε=78.3 for the bulk water environment where the use of $\Delta G_{desolvation}$ is more appropriate.[1,5,7,8] [Fong 2016[1,5],Rayne 2010[7], Rizzo 2006[8]]

When a solvated ligand enters a protein binding cavity and begins to bind with the protein, the desolvation processes involving the protein and ligand may involve extensive rearrangement of water molecules, and possibly some expulsion of water molecules from the cavity. A significant change in solvation entropy should then occur on the side on the ligand and the protein receptor. It is postulated that the $\Delta G_{desolv,CDS}$ values may be a close approximation of how a solvated inhibitor reacts as it approaches and starts to enter the binding pocket (dielectric constant ε=20-30) and leaves the bulk water environment (ε =78.3) and binding starts to occur (ε=7-10). This scenario has been modelled using more intensive molecular mechanics computations.[9,10,11] [Setny 2010[9], Shan 2010[10], Mondial 2014[11]]

A modified form of the general equation 1 using the free energy of water desolvation ($\Delta G_{desolv,CDS}$) and the lipophilicity free energy ($\Delta G_{lipophilicity}$), where CDS represents the first solvation shell solvent properties, may be a good approximation of the drug approaching the entry of the protein receptor pocket or the surface of the protein transporter. Desolvation of water from the drug ($\Delta G_{desolv,CDS}$) before binding in the receptor pocket is required, and hydrophobic interactions between the drug and protein ($\Delta G_{lipo,CDS}$) is a positive contribution to binding.

In this study, the general equation will be applied to the critical steps as shown in Figure 1 as they affect the overall anti-bacterial and anti-malarial properties of the fluoroquinolones FQs, and the potential serious cardiac side effects of FQ-hERG induced QT prolongation and Torsades de Pointes (TdP)

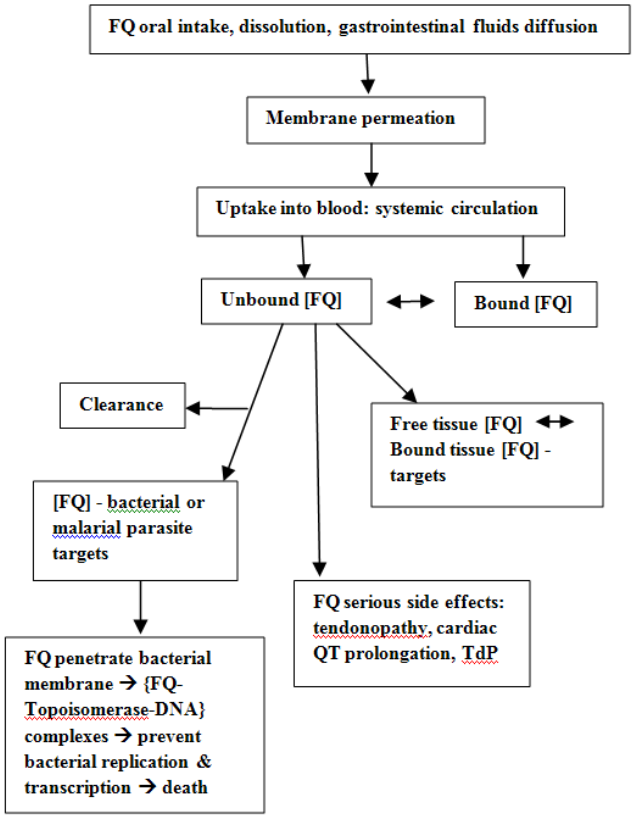


Figure 1. Flow chart of fluoroquinolone FQ processes involved in anti-bacterial or anti-malarial therapy or serious side effects

(a) FQ oral intake, dissolution, gastrointestinal fluid diffusion

Oral administration is the most commonly used drug administration route. The ability to predict the rate and extent of absorption of candidate drugs after oral administration is crucial during the preclinical phase of development. Drug absorption from the gastrointestinal (GI) tract is affected by many factors, including the physiological conditions of the GI tract (including absorptive surface area, local pH, food effects, intestinal transit time, passive intestinal permeability) and the physio-chemical properties of the drug (including solubility, molecular size, polarity, lipophilicity, stability). Intestinal active drug transporters are also involved in controlling oral drug permeability and absorption. The drug intestinal permeability measure P_{eff} is widely used indicator of both the rate and/or extent of drug absorption (F_a) in humans. A good correlation exists between the human jejunal permeability

(P_{eff}) measured using single-pass perfusion techniques and the fraction of dose absorbed from an immediate-release, rapidly dissolving drug.[12-15] [Dahan 2012[12], El-Kattan 2012[13], Hou 2006[14], Waterbeemd 2003[15]]

The solubility of orally administered drugs is a critical rate determining factor in determining bioavailability. FQs are generally fairly insoluble in water, with most modern FQs being zwitterionic, having both a carboxylic acid (pK_a ~5.5-6.3) and basic amine group (pK_a ~ 7.6-9.3). Minimum solubility is around the physiological pH ~ 7.4, where all concentrations of the cationic, neutral, anionic and zwitterionic species vary according to the pK_a of the various FQs. The isoelectric point pI for many FQs is around 6.8-7.6 where the mole fractions of the neutral and zwitterionic species are maximal where their ratio is invariant and pH independent.[16-20] [Sorgel 1993[16], Liu[17], Takacs-Novak 1990[18], Ross 1992[19], Zhang 2014[20]] We have previously shown that the general equation applies to a wide range of equilibrium and kinetic solubilities of drugs. These observed correlations are sensitive to the various charged, neutral and zwitterionic forms of drug species.[1-5] [Fong 2015[1-5]]

The lipophilicity of these FQs can vary according to the pH and pI points. As lipophilicity is a critical factor in drug discovery and pharmacology, the determination is critical. A common used method is logP (neutral species) or logD (ionic species) derived from water-octanol partitioning experiments. LogP measurements of FQ have been made[21] [Takacs-Novak 1992] but a major criticism of logP partitioning is that n-octanol contains 2.8M water at equilibrium, a possible major error for sparingly soluble drugs. $\mathbf{\Delta G_{lipophilicity}}$ calculated from the solvation energy of the drug in n-octane (or n-octanol) is a better measure, and can be applied to neutral, ionized or zwitterionic species quickly and accurately.

It has been concluded that MICs of FQs for *E. coli* (Gram negative) involved mainly neutral and zwitterionic forms (as well as cationic and anionic species). However since drug transport into Gram negative bacteria is dominated by porin transport, this result is not surprising. [Zhang 2014] In one study an amphoteric eburnane alkaloid, it was concluded that the zwitterionic form was the dominant form in membrane penetration because it was the dominant species present, despite the zwitterionic form having far lower lipophilicity (log D) than the neutral form (log P).[22] [Mazak 2012]

(b) FQ membrane permeation: uptake in blood and systemic circulation

Passive membrane diffusion is comprised of two pathways: the paracellular pathway, where the drug diffuses through the aqueous pores at the tight junctions between the intestinal enterocytes, and the trans-cellular pathway, which requires the drug to permeate the cell membrane of the enterocyte. The paracellular pathway is thought to be important for intestinal transport of smaller (MW less than 250) and more hydrophilic drugs which are not repelled by the net negative charge of the junctional complex. The junctions only comprise 0,01% of the surface area of the intestinal membrane, and become progressively tighter moving from the jejunam to the colon. Trans-cellular diffusion is thought to be the major transport pathway (mainly determined by transport across the apical cell membrane) for the uncharged (neutral) and more lipophilic drugs of MW > 300. The vast majority of drug molecules are transported through the transcellular pathway, and the few which rely on the

paracellular pathway of transportation typically have a much lower bioavailability.[13] [El-Kattan 2012[13]]

Active transport processes in enterocytes express several transporters, belonging to the adenosine triphosphate (ATP) binding cassette (ABC) superfamily and the solute carrier (SLC) superfamilies, on the apical and basolateral membranes for the influx or efflux of drugs.

Drugs with a jejunal $P_{eff} > 1.5 \times 10^{-4}$ cm/s are completely absorbed independent of whether passive or active transport mechanisms are involved. Many drugs that are significantly effluxed in vitro have a rapid and complete intestinal absorption (>85%) mediated by passive transcellular diffusion. The jejunal P_{eff} for drugs transported mainly by absorptive carriers (such as peptide and amino acid transporters) can predict the fraction of the dose absorbed as a consequence of the regional expression. The human intestinal epithelium has a large resistance towards large and hydrophilic compounds, and the paracellular route has a low contribution for compounds larger than approximately molecular weight 200.[23] [Lannernas 2007[23]]

Caco-2 cell permeability is often used as a screening tool for assessing drug oral absorption during the early stages of drug development since these cells possess many structural and functional similarities to normal human enterocytes. Caco-2 permeability has generally correlated well with the fraction of the drug absorbed by the intestinal tissue for many drugs in humans. Caco-2 permeability is widely used as a surrogate for human intestinal permeability. Where active influx and efflux transporters are involved, the jujeunal permeability may sometimes still show a dependency with F_a but may show complex segmental permeability for low permeability drugs. Drugs with inherently high intestinal permeability are not affected by intestinal transporters.[12] [Dahan 2012[12]]

Most modern FQs have a high bioavailability, eg Ciprofloxacin 70%, Levofloxacin 99%, Moxifloxacin 86%, Gatifloxacin 95%, Norfloxacin 30-40%, Ofloxacin 98%. However, binding to blood serum proteins eg Ciprofloxacin 30%, Levofloxacin 31%, Moxifloxacin 48%, Gatifloxacin 20%, Gemifloxacin 20% can reduce bioavailability, and methods to predict how new FQ will bind to serum proteins are useful during drug discovery. The negative effect of protein binding on antibiotic activity is well established, and other effects include lower elimination, lower glomerular filtration, volume of distribution and half life hence the lowering time above MIC.[24] [Schmidt 2010[24]]

(c) FQ transport across bacterial membranes

The cell walls of gram-negative bacteria follow a more general structural format than that of gram-positive bacteria, which can vary considerably in different species. Gram negative bacteria possess a lipid-rich outer membrane (as well as a plasma membrane) and a thin peptidoglycan layer Gram-positive bacteria are enshrouded in thicker, more resilient cell walls and a thicker peptidoglycan layer in their cell wall.

There are three major routes across the *outer* membrane: (a) the hydrophobic or bilayer pathway, involving the lipopolysaccahride LPS bilayer, (b) the self-promoted uptake pathway and (c) the hydrophilic or porin pathway involving the water filled trans-membrane proteins (porins).[25] [Hancock 1997[25]] For most clinically used antibiotics the *hydrophilic or porin*

pathway is more important. Studies on porins have shown that: (i) molecules of 500 Da and less diffuse easily through the outer membrane, and molecules up to 700 Da must be flexible to allow diffusion, (ii) the charge or hydrophilicity has a large effect, with neutral species diffusing fastest, cations generally diffusing faster than anions; multiple charged molecules diffusing even slower than single charged species; zwitterions diffused faster than anions (iii) there was an inverse relationship between lipophilicity (from log P partitioning) and diffusion rate.[26-30] [Nikaido 1985[26], Gunn 2001[27], Hancock 1988[28], Ma 1994[29], Wiese 2003[30]] Mutations to the LPS bilayer can increase drug resistance. More importantly, porin deficiency has been associated with increased bacterial resistance in nearly all Gram-negative species involved in hospital-acquired infections.[31-34] [Charrel 1996[31], Yigit 2002[32], Cornaglia 1995[33], Domenech-Sanchez 2003[34]]

Resistance to fluoroquinolones can involve mutations of the target enzyme and changes to microbial cell permeability. Many Gram-negative bacteria contain genes for multiple efflux pumps, used by bacteria as secretion mechanisms for cellular products, and as defence mechanisms against harmful substances present in the environment. *These efflux pumps also function as an active antibiotic accumulation barrier, by which a drug is pumped out faster than it can diffuse into the cell.* The pumps include: the major facilitator superfamily (MFS), the ABC group, the resistance nodulation division family (RND), the multidrug- and toxic compound extrustion family (MATE), and the small (earlier staphylococcus) multidrug resistance (SMR) family. Of these the RND efflux pumps play the most important role in multidrug resistance in all Gram negatives bacteria.

The MRP2 (or cMOAT or ABCC2) transporter is involved in drug disposition and distribution, particularly of organic anions and drug metabolites such as glucuronides. MRP2 is an efflux transporter that is important in drug biliary excretion and renal excretion, and also prevents xenobiotics from penetrating or accumulating in tissues such as lungs and from crossing the placenta. MRP2 is localized to the apical membrane domain of polarized cells such as hepatocytes, renal proximal tubule and intestinal epithelia, and is also present in the gallbladder, bronchi, and placenta. Multiple ABC transporters contribute to the overall active secretion of some fluoroquinolones (Grepafloxacin), and MRP2 has been demonstrated to mediate biliary excretion of some FQs.[35] [Alvarez 2008[35]]

(d) FQ anti-bacterial mechanism

FQs kill bacteria by increasing levels of DNA strand breaks generated by type II topoisomerases. DNA topoisomerases are enzymes that control the topology of DNA in cells. Topoisomerases I and II are involved in the breakage of one or both strands of the DNA, and the passage of one segment of DNA through a break in another. Nearly all bacteria encode two type II topoisomerases, gyrase and topoisomerase IV. DNA gyrase (particularly in Gram negative bacteria) and topoisomerase IV (particularly in Gram positive bacteria) are primary targets for antibacterial agents, including the fluoroquinolones and the coumarins families. DNA gyrase is essential in all bacteria but is not found in humans, so it is an ideal target for antibacterials. The known reactions of topoisomerases include: relaxation and supercoiling, catenation and decatenation, knotting and unknotting. DNA supercoiling activity is unique to gyrase.

It is well known that type II topoisomerases require divalent metal ions in order to cleave DNA. Both type II enzymes are essential for cell survival. Fluoroquinolones (FQ) form a FQ-Topoisomerase II-DNA complex where the enzyme is trapped with the bound DNA. FQ

can also form a complex with Topoisomerase IV utilizing a bridge between the ketone – carboxylate moiety of the FQ to a Mg ion linked to the serine and glutamate residues of the Topoisomerase IV, as shown in Figure 2.[36] [Redgrave 2014[36]] A "water−metal ion bridge" acts as the primary conduit by which clinically relevant quinolones interact with topoisomerase IV. Mutations of the topoisomerase IV residues which form the {FQ-M^{++}-H_2O-Topo IV} bridge are thought to be responsible for resistance to FQs.[37] [Aldred 2014[37]]

(e) Serious side effects of FQs

The long QT syndrome (LQTS) is a disorder of ventricular repolarization that predisposes affected individuals to cardiac arrhythmia and sudden death. It has long been clinically recognized that drug-induced aLQTS is a serious side effect, and the cardiac hERG potassium channels have been related to this QT prolongation. Drugs such as terfenadine (Seldane) cisapride (Propulsid), grepafloxacin (Raxar) and terodiline block the hERG channel to deleterious effect and thus have been withdrawn from the market. Syncope, polymorphic ventricular tachycardia (torsade de pointes or TdP) and sudden cardiac death were particularly associated with terfenadine. Consequently, a hERG assay is now an indispensable step and a high-quality assay must accompany any investigational new drug (IND) application.[38] [Brown 2004[38]] The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals, issued as CHMP/ICH/423/02, was adopted by CHMP in May 2005.

hERG encodes the pore-forming subunit of a delayed rectifier voltage gated K^+ (VGK) channel. These channels are variously referred to as I_{Kr}, hERG, or Kv11.1 hERG forms the major portion of one of the ion channel proteins (the 'rapid' delayed rectifier current I_{Kr}) that conducts potassium (K^+) ions out of the muscle cells of the heart, and this current is critical in correctly timing the return to the resting state (repolarization) of the cell membrane during the cardiac action potential. In the laboratory the heterologously expressed hERG potassium channel comprises 4 identical alpha subunits, which form the channel's pore through the plasma membrane. Each hERG subunit consists of 6 transmembrane alpha helices, numbered S1-S6, a pore helix situated between S5 and S6, and cytoplasmically located N- and C-termini. The S4 helix contains a positively charged arginine or lysine amino acid residue at every 3rd position and is thought to act as a voltage-sensitive sensor, which allows the channel to respond to voltage changes by changing conformations between conducting and non-conducting states (called 'gating'). This channel is also sensitive to drug binding, as well as decreased extracellular potassium levels, both of which can result in decreased channel function and drug-induced (acquired) long QT syndrome.[39] [Vanderberg 2012[39]]

Among the drugs that can cause QT prolongation, the more common ones include antiarrhythmics (especially Class 1A and Class III), anti-psychotic agents, and certain antibiotics (including quinolones and macrolides).[40,41] [Briasoulis 2011[40], Sanguinetti 2006[41]] Although there exist other potential targets for cardiac adverse effects, the vast majority of drugs associated with acquired QT prolongation are known to interact with the hERG potassium channel. One of the main reasons for this phenomenon is the larger inner vestibule of the hERG channel, thus providing more space for many different drug classes to bind and block this potassium channel.[42] [Milnes 2003[42]]

Fluoroquinolones prolong the QT interval by blocking voltage-gated potassium channels, especially the rapid component of the delayed rectifier potassium current I_{Kr}, expressed by hERG (the human ether-a-go-go-related gene). Moxifloxacin carries the greatest risk of QT

prolongation from all available quinolones in clinical practice and it should be used with caution in patients with predisposing factors for Torsades de Pointes (TdP). Gemifloxacin, levofloxacin, and ofloxacin are associated with a lower risk of QT prolongation compared with Moxifloxacin, and Ciprofloxacin appears to be associated with the lowest risk for QT prolongation and the lowest TdP rate. The overall risk of TdP is small with the use of fluoroquinolones. Clinicians can minimize that risk by avoiding prescriptions of multiple medications associated with QT-interval prolongation, especially in high-risk patients.[40] [Briasoulis 2011[40]]

Materials and methods: Experimental methods

All calculations were carried out using the Gaussian 09 package at the B3LYP/6-31 G*(6d, 7f) level of theory with optimised geometries in water, as this level has been shown to give accurate electrostatic atomic charges, and was used to optimize the IEFPCM/SMD solvent model. With the 6-31G* basis set, the SMD model achieves mean unsigned errors of 0.6 - 1.0 kcal/mol in the solvation free energies of tested neutrals and mean unsigned errors of 4 kcal/mol on average for ions.[6] [Marenich 2009[6]]The 6-31G** basis set has been used to calculate absolute free energies of solvation and compare these data with experimental results for more than 500 neutral and charged compounds. The calculated values were in good agreement with experimental results across a wide range of compounds.[7,8] [Rayne 2010[7],Rizzo 2006[8]] Adding diffuse functions to the 6-31G* basis set (ie 6-31^{+}G**) had no significant effect on the solvation energies with a difference of less than 1% observed in solvents, which is within the literature error range for the IEFPCM/SMD solvent model.[6] [Marenich 2009[6]] It is noted that high computational accuracy for each species in different environments is not the focus of this study, but comparative differences between various species is the aim of the study. The use of various literature values for K_m, IC_{50} to develop the multiple regression equations have much higher uncertainties than the calculated molecular properties. The statistical analyses include the multiple correlation coefficient R^2, the F test of significance, standards errors for the estimates (SEE) and each of the variables SE($\Delta G_{desolvation}$), SE($\Delta G_{lipophilicity}$), SE(Dipole Moment), SE (Molecular Volume), SE(Molecular Volume), as calculated from "t" distribution statistics. Residual analysis was used to identify outliers. The solvation energies, lipophilicities, dipole moments, molecular volumes of the statins are shown in Table 2.

It is noted that the various equations derived in this study are more indicative rather than highly robust in that statistical precision is taken as the goodness of fit (rather than extensive statistical validation) of the experimental data since insufficient experimental data are not available for highly robustly validated outcomes using the four independent variables. The correlations have used data from a single source where the experimental precision is consistent, as compared to using experimental data from multiple sources, where the interlaboratory experimental error can be very large, even for the same cell line, up to a factor of 10 as found by Aronov[63]. However the fact that the different biological processes in this study (Figure 1) are all well correlated by the general equation indicates that the general equation may a universally valid model for biological processes.

Results

Anti-bacterial properties of FQs

Minimum inhibitory concentrations (MIC, μg/ml**,** DM4100 *E. coli*) for 17 FQ drugs: Sarafloxacin 0.086, Sparfloxacin 0.125, Fleroxacin 0.141, Orbifloxacin 0.150, Sitafloxacin 0.015, Gemifloxacin 0.086, Grepafloxacin 0.016, Besifloxacin 0.100, Tosufloxacin 0.050, Lomefloxacin 0.172, Ulifloxacin 0.022, 8-Ethoxy-Moxifloxacin 0.200, Anthofloxacin 0.15, N-Methyl-Gatifloxacin 0.065, PD161144 0.096, Clinafloxacin 0.0095, Balofloxacin 0.484 all as *zwitterionic* species: [Schwanz 2012[43]] **Equation 1**

Log MIC = - 0.049 $\Delta G_{desolvation}$ + 0.142 $\Delta G_{lipophilicity}$ + 0.121 Dipole Moment + **0.009 Molecular Volume** + 0.902

Where R^2 = 0.624, SEE = 0.325, SE($\Delta G_{desolvation}$) = 0.030, SE($\Delta G_{lipophilicity}$) = 0.073, SE(Dipole Moment) = 0.062, SE(Molecular Volume) = 0.003, F = 5.0, Significance F = 0.013

It is noted that treating the FQs as neutral species for the *E.coli* MICs gave a very poor correlation.

Anti-malarial properties of FQs

Plasmodium is a genus of parasitic protozoa, many of which cause malaria in their hosts. Antimalarial activities (IC_{50}) of 20 quinolones and fluoroquinolones (Moxifloxacin, Lomefloxacin, Enoxacin, Norfloxacin, Grepafloxacin, Ciprofloxacin, Gatifloxacin, Clinifloxacin, Fleroxacin, Flumequine, Nalidixic Acid, Pipemidic Acid, Piromidic Acid, Chloroquine, Rufloxacin, Trovafloxacin (all as *neutral* species), Pefloxacin zwitterion (ZW), Sparflox (ZW), Levoflocacin (ZW), Ofloxacin (ZW) against **chloroquine-sensitive** (3D7) strains of *Plasmodium falciparum* in vitro [Mahmoudi 2003[44]] **Equation 2**

IC_{50} = 4.96 $\Delta G_{desolvation}$ + 7.10 $\Delta G_{lipophilicity}$ - 3.27 Dipole Moment - 0.26 Molecular Volume + 135.55

Where R^2 = 0.656, SEE = 20.18, SE($\Delta G_{desolvation}$) = 2.37, SE($\Delta G_{lipophilicity}$) = 7.16, SE(Dipole Moment) = 2.69, SE(Molecular Volume) = 0.23, F=7.16, Significance F = 0.002, n=20

Antimalarial activities (IC_{50}) of 20 quinolones and fluoroquinolones (Moxifloxacin, Lomefloxacin, Enoxacin, Norfloxacin, Grepafloxacin, Ciprofloxacin, Gatifloxacin, Clinifloxacin, Fleroxacin, Flumequine, Nalidixic Acid, Pipemidic Acid, Piromidic Acid, Chloroquine, Rufloxacin, Trovafloxacin (all as *neutral* species), Pefloxacin zwitterion (ZW), Sparflox (ZW), Levoflocacin (ZW), Ofloxacin (ZW) against **chloroquine-resistant** (NF54-R) strains of *Plasmodium falciparum* in vitro [Mahmoudi 2003[44]] **Equation 3**

IC_{50} = 2.42 $\Delta G_{desolvation}$ + 5.84 $\Delta G_{lipophilicity}$ - 0.29 Dipole Moment - 0.06 Molecular Volume + 60.63

Where R^2 = 0.481, SEE = 17.53, SE($\Delta G_{desolvation}$) = 1.42, SE($\Delta G_{lipophilicity}$) = 4.30, SE(Dipole Moment) = 1.61, SE(Molecular Volume) = 0.14, F=3.47, Significance F = 0.033, n=20

The correlation equations for the 3D7 and NF54-R *Plasmodium falciparum* strains were very similar if Pefloxacin, Sparflox, Levoflocacin, and Ofloxacin were omitted as large outliers (R^2 0.525, SE 28.65, F 2.77, F significance 0.087 for the 3D7 correlation, and R^2 0.485, SE 213.07, F 2.35, F significance 0.124 for the NF54-R correlation); however including these four FQs as the zwitterionic species gave the two equation 2 and 3 above which are better statistical correlations. This result shows that these correlations can differentiate between the neutral and zwitterionic forms of the FQs in their ability to inhibit *Plasmodium falciparum*

strains. This observation strongly suggests that it is mainly the neutral form of the FQs that determines the <u>*overall*</u> efficacy against the *Plasmodium falciparum* strains, despite the many steps that must be involved in FQ pharmacokinetics for the FQ to achieve their anti-malarial efficacy.

Antimalarial activities of 10 quinolones and fluoroquinolones (Norfloxacin, Ciprofloxacin, Clinifloxacin, Piromidic Acid, Rufloxacin, Trovafloxacin, Sparfloxacin (all as *neutral* species), Pefloxacin ZW, Ofloxacin ZW, Grepafloxacin ZW) against hepatic stages of *P. yoelii yoelii* at 48 h. [Mahmoudi 2003[44]] **Equation 4**

$$\mathbf{IC_{50} = 0.92\ \Delta G_{desolvation} - 5.32\ \Delta G_{lipophilicity} - 4.56\ Dipole\ Moment + 1.64\ Molecular\ Volume} - 288.26$$

Where $R^2 = 0.756$, SEE = 18.90, SE($\Delta G_{desolvation}$) = 2.25, SE($\Delta G_{lipophilicity}$) = 6.17, SE(Dipole Moment) = 2.64, SE(Molecular Volume) = 0.433, F=3.87, Significance F = 0.085, n=10

Antimalarial activities of 10 quinolones and fluoroquinolones (Norfloxacin, Ciprofloxacin, Clinifloxacin, Piromidic Acid, Rufloxacin, Trovafloxacin, Sparfloxacin (all as *neutral* species), Pefloxacin ZW, Ofloxacin ZW, Grepafloxacin omitted) against hepatic stages of *P. yoelii yoelii* at 48 h. [Mahmoudi 2003[44]] **Equation 4a**

$$\mathbf{IC_{50} = -0.27\ \Delta G_{desolvation} - 8.28\ \Delta G_{lipophilicity} - 3.96\ Dipole\ Moment + 1.18\ Molecular\ Volume} - 288.26$$

Where $R^2 = 0.767$, SEE = 17.70, SE($\Delta G_{desolvation}$) = 2.30, SE($\Delta G_{lipophilicity}$) = 6.21, SE(Dipole Moment) = 2.52, SE(Molecular Volume) = 0.53, F=3.30, Significance F = 0.137, n=9

Comparison of eq 4 with eq 4a shows that by including Grepafloxacin (which showed the greatest effect against *P. yoelii yoelii*) as a zwitterion is a better statistical correlation than by simply omitting Grepafloxacin as an outlier from eq 4a. This outcome gives confidence that these correlations can distinguish amongst the zwitterionic and neutral forms of the various FQs in exerting their anti-malarial effect.

Ring stage parasitized erythrocytes were used for the 3D7 and NF54-R *Plasmodium falciparum* sudies. Mouse or human hepatocyte cultures were incubated for 24 h before sporozoite inoculation for the *P. yoelii yoelii* studies.[44] [Mahmoudi 2003[44]] Comparison of eqs 2,3 with eq 4 show that the effect of the anti-malarials on the erythrocytes is very different from the effect on the hepatocytes with a large sensitivity on the lipophilicity and dipole moment (and lesser effect on the desolvation) for the hepatocytes. This observation is consistent with the more lipophilic environment in the liver tissue than for the red blood cells where a far more hydrophilic environment exists.

Chronic bacterial prostatis (CBP)

CBP is mainly caused by Gram-negative (and some Gram-positive) uropathogens, and FQs are the drugs of choice because of the favourable pharmacokinetics and anti-microbial spectrum. The concentrations of FQs in human seminal and prostatic fluids (and prostatic tissue) are important guides to efficacy of the FQs against CBP.[45] [Weidner 2008[45]] The median concentrations in prostatic fluid:plasma ratios (normalized to a 400 mg dose) for Moxifloxacin, Lomefloxacin, Enoxacin, Norfloxacin, Ciprofloxacin, Gatifloxacin, Levofloxacin, Ofloxacin, all treated as *neutral* species (Fleroxacin omitted as outlier). All FQs were orally administered except Ciprofloxacin and Ofloxacin were IV. **Equation 5**

Prostatic fluid:plasma = - 0.31 $\Delta G_{desolvation}$ - 0.27 $\Delta G_{lipophilicity}$ + 0.21 Dipole Moment - 0.01 Molecular Volume + 2.95

Where R^2 = 0.933, SEE = 0.21, SE($\Delta G_{desolvation}$) = 0.06, SE($\Delta G_{lipophilicity}$) = 0.07, SE(Dipole Moment) = 0.11, SE(Molecular Volume) = 0.01, F=10.52, Significance F = 0.041, n=8

FQ uptake into rat brains and brain:blood plasma concentrations

In vivo study of the steady state uptake (R_{ss}) of FQ into rat brains and the ratio of brain to blood plasma concentrations (F) were determined for gatifloxacin, ofloxacin, ciprofloxacin, lomefloxacin, rufloxacin, pazufloxacin, norfloxacin, prulifloxacin, balofloxacin, caderofloxacin as neutral species, and sparfloxacin as the zwitterion. Sparfloxin was a clear outlier from the correlations with the *neutral* FQs, and omitting sparfloxacin or including it as a zwitterion gave similar statistical results. Treating all species as zwitterions gave a very poor correlation. [Liu 2005[46]] **Equation 6**

R_{ss} = -12.1$\Delta G_{desolvation}$ – 31.2$\Delta G_{lipophilicity}$ +12.5 Dipole Moment + 0.7 Molecular Volume – 367.0

Where R^2 = 0.858, SEE = 88.9, SE($\Delta G_{desolvation}$) = 11.2, SE($\Delta G_{lipophilicity}$) = 41.1, SE(Dipole Moment) = 28.5, SE(Molecular Volume) = 1.9, F=9.05, Significance F = 0.010

Equation 7

F = - 0.017$\Delta G_{desolvation}$ +0.010$\Delta G_{lipophilicity}$ +0.040 Dipole Moment + 0.000 Molecular Volume – 367.0

Where R^2 = 0.754, SEE = 0.061, SE($\Delta G_{desolvation}$) = 0.007, SE($\Delta G_{lipophilicity}$) = 0.028, SE(Dipole Moment) = 0.020, SE(Molecular Volume) = 0.001, F=4.59, Significance F = 0.048

FQ oral intake, dissolution, gastrointestinal fluid diffusion

Water solubility data (internally consistent very accurate equilibrium and kinetic solubility data base from the Goodman laboratory[47]) for 9 closely structurally related FQs was examined, ranging from Difloxacin 251.311 to Danofloxacin 1260 μM for 8 drugs, but with a very high solubility (apparent outlier) for Oxfloxacin 54262.5 μM [Goodman[46]]. There was a good correlation when treating the drugs as neutral species, with Ofloxacin being a clear outlier unless treated as a zwitterion. Treating all FQs as zwitterions gave a fairly poor correlation R^2 0.621, F significance 0.32). However, the best correlation was obtained when treating the drugs as anions, and Oxfloxocin as a zwitterion.

Equilibrium solubility of 9 floxacins: Sarafloxacin, Norfloxacin, Difloxacin, Enrofloxacin, Lomefloxacin, Ciprofloxacin, Sparfloxacin, Danofloxacin treated as *anionic* species, Ofloxacin as zwitterion **Equation 8**

Log solubility = 0.054 $\Delta G_{solvation}$ + 0.270 $\Delta G_{lipophilicity}$ + 0.165 Dipole Moment - 0.009 Molecular Volume + 13.420

Where R^2 = 0.921, SEE = 0.293, SE($\Delta G_{solvation}$) = 0.062, SE($\Delta G_{lipophilicity}$) = 0.115, SE(Dipole Moment) = 0.036, SE(Molecular Volume) = 0.005, F=11.61, Significance F = 0.018

Equilibrium solubility of 9 floxacins: Sarafloxacin, Norfloxacin, Difloxacin, Enrofloxacin, Lomefloxacin, Ciprofloxacin, Sparfloxacin, Danofloxacin treated as *neutral* species, Ofloxacin as zwitterion **Equation 9**

Log solubility = 0.085 $\Delta G_{solvation}$ + 0.188 $\Delta G_{lipophilicity}$ - 0.006 Dipole Moment - 0.009 Molecular Volume + 5.684

Where R^2 = 0.838, SEE = 0.419, SE($\Delta G_{solvation}$) = 0.121, SE($\Delta G_{lipophilicity}$) = 0.170, SE(Dipole Moment) = 0.172, SE(Molecular Volume) = 0.010, F5.56, Significance F = 0.070

Equilibrium solubility of 9 floxacins: Sarafloxacin, Norfloxacin, , Difloxacin, Enrofloxacin, Lomefloxacin, Ciprofloxacin, Sparfloxacin, Danofloxacin treated as *anionic* species, Ofloxacin as zwitterion, **Equation 10**

Log solubility = 0.035 ΔG_{CDS} + 0.344 $\Delta G_{lipophilicity}$ + 0.142 Dipole Moment - 0.007 Molecular Volume + 11.512

Where R^2 = 0.914, SEE = 0.306, SE($\Delta G_{solvation}$) = 0.056, SE($\Delta G_{lipophilicity}$) = 0.105, SE(Dipole Moment) = 0.026, SE(Molecular Volume) = 0.005, F=10.61, Significance F = 0.021

Equations 11 and 12 show the same floxacin data analysed using kinetic solubility.[47] [Goodman] Kinetic solubility is the concentration of a compound in solution when an induced precipitate first appears, whereas equilibrium solubility is the concentration of compound in a saturated solution when excess solid is present, and solution and solid are at equilibrium. Kinetic solubility is commonly used in early drug discovery protocols. Equations 11 and 12 are very similar to those for eq 8,9 and 10.

Kinetic solubility of 9 floxacins: Sarafloxacin, Norfloxacin, Difloxacin, Enrofloxacin, Lomefloxacin, Ciprofloxacin, Sparfloxacin, Danofloxacin treated as *anionic* species, Ofloxacin as zwitterion **Equation 11**

Log solubility = 0.053 $\Delta G_{solvation}$ + 0.226 $\Delta G_{lipophilicity}$ + 0.130 Dipole Moment - 0.014 Molecular Volume + 14.806

Where R^2 = 0.709, SEE = 0.524, SE($\Delta G_{solvation}$) = 0.111, SE($\Delta G_{lipophilicity}$) = 0.206, SE(Dipole Moment) = 0.065, SE(Molecular Volume) = 0.009, Significance F = 0.204

Kinetic solubility of 9 floxacins: Sarafloxacin, Norfloxacin, Difloxacin, Enrofloxacin, Lomefloxacin, Ciprofloxacin, Sparfloxacin, Danofloxacin treated as *anionic* species, Ofloxacin as zwitterion **Equation 12**

Log solubility = 0.167ΔG_{CDS} + 0.372$\Delta G_{lipophilicity}$ + 0.106Dipole Moment - 0.014 Molecular Volume + 11.512

Where R^2 = 0.906, SEE = 0.299, SE($\Delta G_{solvation}$) = 0.056, SE($\Delta G_{lipophilicity}$) = 0.102, SE(Dipole Moment) = 0.025, SE(Molecular Volume) = 0.004, Significance F = 0.025

FQ human intestinal permeability, renal organic anion transporters, BBB permeability

Avdeef[48] and Wang[49] have sought to calculate human jejunal permeability and absorption from experimental Caco-2 data, assuming passive diffusion (transcellular and paracelloular). Intestinal Jejunal permeability Caco-2 pH 6.5 for 14 fluoroquinolones: Sarafloxacin, Norfloxacin, Ofloxacin, Difloxacin, Enrofloxacin, Lomefloxacin, Ciprofloxacin, Sparfloxacin, Danofloxacin, Gatifloxacin, Grepafloxacin, Pefloxacin, Flumequine, Moxifloxacin treated as *neutral* species. Log values are negative. Correlation with all FQ as ionic species was much worse F significance 0.604. Avdeef 2010[48], Wang 2014[49] **Eq 13**

Log Caco-2 permeability = -0.274 $\Delta G_{desolvation}$ - 0.268 $\Delta G_{lipophilicity}$ + 0.22 Dipole Moment - 0.012 Molecular Volume - 2.450

Where R^2 = 0.600, SEE = 0.616, SE($\Delta G_{solvation}$) = 0.108, SE($\Delta G_{lipophilicity}$) = 0.185, SE(Dipole Moment) = 0.126, SE(Molecular Volume) = 0.015, F=3.4, Significance F = 0.061

Calculated human jejunal permeability rates (passive permeabilities 10^{-4} cm/s) from experimental Caco-2 values (pH 6.5), from Avdeef 2010[48] and Wang 2014[49] for the 14 FQ treated as *neutrals* species. Log permeabilities are negative values. **Equation 14**

Log calculated human permeability rates = -0.075 $\Delta G_{desolvation}$ - 0.107 $\Delta G_{lipophilicity}$ + 0.059 Dipole Moment - 0.004 Molecular Volume - 3.251

Where R^2 = 0.436, SEE = 0.243, SE($\Delta G_{solvation}$) = 0.042, SE($\Delta G_{lipophilicity}$) = 0.073, SE(Dipole Moment) = 0.050, SE(Molecular Volume) = 0.006, F=1.75, Significance F = 0.225

Since PAMPA membranes have no transporter capability, whereas Caco-2 membranes do have transporter capability, a comparison between PAMPA and Caco-2 transport of FQs may shed light on their intestinal permeability.

PAMPA intrinsic permeability coefficients (P_e 10^{-6} cm/s) for the *uncharged* forms of 4′N-R-norfloxacin: 0.7 (R=H), 49 (Me), 132 (*n*-Pr), 365 (*n*-Bu); 4′N-R-ciprofloxacin: 2.7 (H), 37 (Me), 137 (*n*-Pr), 302 (*n*-Bu); 4′N-R-3′-methylciprofloxacin: 3.8 (H), 20 (Me), 51 (Et), 160 (*n*-Pr), 418 (*n*-Bu). The alkyl chain length at the 4′N-position of the piperazine residue was varied as a function of pH from 4 to 10. Rat in situ permeability measurements were correlated with the PAMPA P_e measurements (r^2=0.87).[50] [Barmejo 2004[50]] The substitution of various alkyl groups at the 4′N-position of the piperazine is related to a secondary FQ-gyrase a binding mode that may overcome gyrase A mediated FQ resistence.[51] [Malik 2016[51]]

Equation 15

Log PAMPA P_e = - 0.58$\Delta G_{desolvation}$ - 0.12$\Delta G_{lipophilicity}$ -0.91Dipole Moment + 0.01 Molecular Volume (Water) + 2.339

Where R^2 = 0.764, SEE = 0.52, SE($\Delta G_{desolvation}$) = 0.39, SE($\Delta G_{lipophilicity}$) = 0.36, SE(Dipole Moment) = 0.71, SE(Molecular Volume) = 0.005, F = 6.46, Significance F = 0.012

Equation 16

Log PAMPA P_e = 178.1$\Delta G_{desol,CDS}$ + 178.5$\Delta G_{lipophilicity}$ -210.7Dipole Moment + 900.9 Molecular Volume (Water) – 8305.2

Where R^2 = 0.856, SEE = 67.3, SE($\Delta G_{desolvation}$) = 84.1, SE($\Delta G_{lipophilicity}$) = 76.4, SE(Dipole Moment) = 47.8, SE(Molecular Volume) = 234.5, F = 11.85, Significance F = 0.002

Transport of FQs by renally expressed basolateral SLC22 super family transporters: mOAT (mouse organic anion transporter mOAT3, active in adsorption and excretion of anions). Enoxacin, fleroxacin, levofloxacin, lomefloxacin, moxifloxacin, prulifloxacin, sparfloxacin*, norfloxacin, and ciprofloxacin* exhibit competitive inhibition for mOat3 with K_i = 396, 817, 515, 539, 1356, 299, 205, 558 and 198 μM respectively.[52] [Mulgaonkar 2012[52]] A very poor correlation (R^2 0.187, F significance 0.908) was obtained using the anionic FQ species, but Ciprofloxacin and Sparfloxacin were clear outliers. A better correlation was found using all species as zwitterions (R^2 0.517, F significance 0.474), but again Ciprofloxacin and Sparfloxacin were clear outliers. Treating all species as zwitterions but with Ciprofloxacin* and Sparfloxacin* treated as anions gave a vastly improved correlation as shown below.

Competitive basolatal renal inhibition of mOAT3 for 9 FQ drugs,[52] [Mulgaonkar 2012[52]] all drugs as *zwitterionic* species, or anionic species* (Ciprofloxacin*, Sparfloxacin*): **Eq 17**

Log K_i = -0.045 $\Delta G_{desolvation}$ - 0.007 $\Delta G_{lipophilicity}$ + 0.052 Dipole Moment + 0.001 Molecular Volume + 4.184

Where R^2 = 0.849, SEE = 0.149, SE($\Delta G_{desolvation}$) = 0.023, SE($\Delta G_{lipophilicity}$) = 0.060, SE(Dipole Moment) = 0.014, SE(Molecular Volume) = 0.002, F = 5.62, Significance F = 0.061

Steady state in vitro competitive uptake of ^{14}C Grepafloxacin in rat brain capillary endothelial cells in the presence of bicarbonate ions by 7 FQ drugs: Levofloxacin, Sparfloxacin, Nalidixic Acid as *ionic* species, Enofloxacin, Norfloxacin, Ofloxacin. Grepafloxacin as zwitterionic species: [Tamai 2000[53]] **Equation 18**

Log K_i = -0.009 $\Delta G_{desolvation}$ - 0.097 $\Delta G_{lipophilicity}$ - 0.011 Dipole Moment - 0.004 Molecular Volume + 0.653

Where R^2 = 0.880, SEE = 0.100, SE($\Delta G_{desolvation}$) = 0.019, SE($\Delta G_{lipophilicity}$) = 0.044, SE(Dipole Moment) = 0.010, SE(Molecular Volume) = 0.002, F=3.67, Significance F = 0.225

The in vivo brain distribution of the FQ HSR-903 (Olamufloxacin), grepafloxacin and possibly other FQ were strongly influenced by efflux transporters, including P-gp at the BBB. The effect of 9 FQ on steady state uptake (%) of HSR-903 on in vitro primary-cultured monolayers of brain capillary endothelial cells was studied.

Effect on in vitro brain capillary endothelial cell uptake of HSR-903 (Olamufloxacin) by the following FQs: Grepafloxacin 360.5, Sparfloxacin 262.9, Levofloxacin 137.2, Tosufloxacin 120.1, Lomefloxacin 137.0, Ofloxacin 130.5, Enoxacin 190.1, Norfloxacin 253.2, all *neutral* species, Nalidixic acid 50.9 was an outlier when treated as a neutral species, and gave an equally good correlation when omitted as an outlier or included as an ionic species. The correlation when treating all FQ species as zwitterions or ions was very poor. [Tamai and Tsuji 2000[54]]

Equation 19

Uptake % = -46.45$\Delta G_{desolvation}$ – 5.14$\Delta G_{lipophilicity}$ +150.95 Dipole Moment - 8.34 Molecular Volume + 1389.1

Where R^2 = 0.925, SEE = 36.48, SE($\Delta G_{desolvation}$) = 7.90, SE($\Delta G_{lipophilicity}$) = 11.87, SE(Dipole Moment) = 26.27, SE(Molecular Volume) = 1.56, F=12.4, Significance F = 0.015

FQ binding to plasma proteins

Bovine serum albumin BSA has been used as a proxy for human serum albumin – drug binding interactions. The binding constants for eight fluoroquinolone antibiotics (ciprofloxacin, enoxacin, fleroxacin, levofloxacin, lomefloxacin, norfloxacin, ofloxacin, pefloxacin) and bovine serum albumin (BSA) are known, (K_b 10^4 M^{-1}).[55] [Liu 2006[55]] All FQs in *zwitterionic* form.

Equation 20

K_b = - 0.97 $\Delta G_{desolvation}$ - 2.50 $\Delta G_{lipophilicity}$ - 0.74 Dipole Moment + 0.05 Molecular Volume + 35.55

Where R^2 = 0.943, SEE = 0.97, SE($\Delta G_{desolvation}$) = 0.23, SE($\Delta G_{lipophilicity}$) = 0.58, SE(Dipole Moment) = 0.44, SE(Molecular Volume) = 0.02, F=8.20, Significance F = 0.111, n=8

Equation 21

K_b = - 0.95 $\Delta G_{desol,CDS}$ - 0.02 $\Delta G_{lipophilicity}$ - 0.06 Dipole Moment - 0.002 Molecular Volume + 0.35

Where R^2 = 0.974, SEE = 0.55, SE($\Delta G_{desolvation}$) = 0.13, SE($\Delta G_{lipophilicity}$) = 0.22 SE(Dipole Moment) = 0.25, SE(Molecular Volume) = 0.018, F=25.58, Significance F = 0.010, n=8

The correlations using the neutral forms of the FQs were very poor.

FQ serious side effects: hERG cardiac QT prolongation

Hagiwara 2001[56] has assessed the effects of 10 fluoroquinolones on cardiac repolarization by measuring the action potentials recorded from isolated guinea pig right ventricular myocardia under constant conditions, namely, myocardial preparations were electrically stimulated at the fixed pacing frequency of 1 Hz. Sparfloxacin was the most active drug, at 10 μM, prolonged APD_{90} 8.4%, and APD_{50} 9.0%. All other drugs were measured at 100 μM as the APD_{90} values. Blockade of I_{Kr} leads to a delay in the cardiac repolarization and prolongs the

APD of myocardia and consequently prolongs the QT interval on the ECG. The action potential duration (APD_{90} 100 μM) recorded from isolated guinea pig ventricular myocardia from ten fluoroquinolone antibacterial agents: Sparfloxacin 40.8, Moxifloxacin 25.1, Grepafloxacin 23.8, Gatifloxacin 12.7, Ciprofloxacin 3.3, Trovafloxacin 2.9, Levofloxacin 0.8 (all as *neutral* species), Tosufloxacin ZW 5.2, Gemifloxacin ZW 4.2, Sitafloxacin ZW 2.4% [Hagiwara 2001[56]]

Equation 22

APD_{90} = - 2.13 $\Delta G_{desolvation}$ – 7.80 $\Delta G_{lipophilicity}$ - 1.29 Dipole Moment - 0.37 Molecular Volume - 288.26

Where R^2 = 0.611, SEE = 11.24, SE($\Delta G_{desolvation}$) = 1.51, SE($\Delta G_{lipophilicity}$) = 4.08, SE(Dipole Moment) = 1.38, SE(Molecular Volume) = 0.25, F=1.96, Significance F = 0.238, n=10

Equation 23

APD_{90} = 3.49 $\Delta G_{desol,CDS}$ – 4.26 $\Delta G_{lipophilicity}$ - 3.23 Dipole Moment - 0.39 Molecular Volume - 288.26

Where R^2 = 0.735, SEE = 9.28, SE($\Delta G_{desolvation}$) = 1.52, SE($\Delta G_{lipophilicity}$) = 1.75, SE(Dipole Moment) = 1.17, SE(Molecular Volume) = 0.20, F=3.47, Significance F = 0.101, n=10

Kang 2001[57] has examined the effect of FQs on chinese hamster ovary cells (CHO cells) transfected with cDNA encoding the hERG K_1 channel cloned from a human neuroblastoma cell line. The inhibition (IC_{50} μM) of the hERG cardiac K^+ channel by the FQs Sparfloxacin 18, Moxifloxacin 129, Grepafloxacin 50, Gatifloxacin 130, Ciprofloxacin 966, Levofloxacin 815, Ofloxacin 1420 (as zwitterion) [Kang 2001[57]] **Equation 24**

IC_{50} = 210.47 $\Delta G_{desolvation}$ + 334.81 $\Delta G_{lipophilicity}$ - 160.22 Dipole Moment + 17.71 Molecular Volume - 1588.41

Where R^2 = 0.986, SEE = 116.61, SE($\Delta G_{desolvation}$) = 30.51, SE($\Delta G_{lipophilicity}$) = 70.01, SE(Dipole Moment) = 67.23, SE(Molecular Volume) = 3.15 F=35.21, Significance F = 0.027, n=7

The influence of the peak free plasma concentrations of the FQ on the IC_{50} values has also been investigated. The inhibition (IC_{50} μM/[Plasma]) of the hERG cardiac K^+ channel by the FQs Sparfloxacin 18, Moxifloxacin 129, Grepafloxacin 50, Gatifloxacin 130, Ciprofloxacin 966, Levofloxacin 815, Ofloxacin 1420 (as zwitterion) [Kang 2001[57]] **Equation 25**

IC_{50} /[Plasma] = 17.35 $\Delta G_{desolvation}$ + 23.97 $\Delta G_{lipophilicity}$ - 15.23 Dipole Moment + 1.24 Molecular Volume - 1588.41

Where R^2 = 0.971, SEE = 14.30, SE($\Delta G_{desolvation}$) = 3.74, SE($\Delta G_{lipophilicity}$) = 8.58, SE(Dipole Moment) =8.24, SE(Molecular Volume) = 0.38 F=16.51, Significance F = 0.057, n=7

Eqs 24 and 25 using **$\Delta G_{desolvation}$** where the IC_{50} values are corrected for the peak plasma concentrations of the FQs are very similar except the coefficients are roughly 12 times less sensitive for eq 25.

The inhibition (IC_{50} μM) of the hERG cardiac K^+ channel by the FQs Sparfloxacin 18, Moxifloxacin 129, Grepafloxacin 50, Gatifloxacin 130, Ciprofloxacin 966, Levofloxacin 815 (as Zwitterion), Ofloxacin 1420 (as zwitterion) [Kang 2001[57]] **Equation 26**

IC_{50} = 140.64 $\Delta G_{desol,CDS}$ - 4.85 $\Delta G_{lipophilicity}$ + 21.50 Dipole Moment + 0.49 Molecular Volume + 780.38

Where R^2 = 0.965, SEE = 183.91, SE($\Delta G_{desolvation}$) = 45.77, SE($\Delta G_{lipophilicity}$) = 95.09, SE(Dipole Moment) = 55.59, SE(Molecular Volume) = 7.04, F=13.86, Significance F = 0.067, n=7

The influence of the peak free plasma concentrations of the FQs on the IC_{50} values has also been investigated. The inhibition (IC_{50} μM/[Plasma]) of the hERG cardiac K^+ channel by the FQs Sparfloxacin 18, Moxifloxacin 129, Grepafloxacin 50, Gatifloxacin 130, Ciprofloxacin 966, Levofloxacin 815 (as Zwitterion), Ofloxacin 1420 (as zwitterion) [Kang 2001[57]] **Eq 27**

IC_{50} /[Plasma] = 13.07 $\Delta G_{desol,CDS}$ + 0.77 $\Delta G_{lipophilicity}$ + 2.46 Dipole Moment + 0.13 Molecular Volume + 70.48

Where R^2 = 0.966, SEE = 15.48, SE($\Delta G_{desolvation}$) = 3.85 SE($\Delta G_{lipophilicity}$) = 8.00, SE(Dipole Moment) = 4.68, SE(Molecular Volume) = 0.59, F=14.00, Significance F = 0.068, n=7

Eqs 26 and 27 using **$\Delta G_{desol,CDS}$** where the IC_{50} values are corrected for the peak plasma concentrations of the FQs are very similar except the coefficients are roughly 11 times less sensitive for eq 27

FQ anti-bacterial mechanism

Inhibitory concentrations (IC_{50}, μM**,** inhibition of gyrase supercoiling activity) for 12 FQ and Q drugs: Difloxacin, Enofloxacin, Norfloxacin, Pefloxacin. Ciprofloxacin, 8-Fluoro-Ciprofloxacin, 8-Chloro-Ciprofloxacin, 8-Bromo-Ciprofloxacin, 8-Ethoxy-Ciprofloxacin, Oxalinic Acid, Piromidic Acid, Pipemidic Acid, as *anionic* species. (Ofloxacin was omitted as an outlier, which is consistent with Ofloxacin being a racemic mixture, with the S isomer having twice the anti-bacterail activity of the R isomer[58] [Morrissey 1996[58]]). Ciprofloxacin and its 8-substituted derivatives, (8-F, 8-Cl, 8-Br, 8-Ethoxy) had the lowest IC50s (the most potent), and the nonfluorinated compounds oxolinic acid, pipemidic acid, and piromidic acid had the highest IC50s. [Noble 2003[59]] **Equation 28**

Log IC_{50} = - 0.007 $\Delta G_{desolvation}$ - 0.195 $\Delta G_{lipophilicity}$ - 0.058 Dipole Moment - 0.014 Molecular Volume - 0.816

Where R^2 = 0.753, SEE = 0.427, SE($\Delta G_{desolvation}$) = 0.056, SE($\Delta G_{lipophilicity}$) = 0.093, SE(Dipole Moment) = 0.089, SE(Molecular Volume) = 0.006, Significance F = 0.027

Equation 29

Log IC_{50} = 0.083 ΔG_{CDS} - 0.217 $\Delta G_{lipophilicity}$ - 0.100 Dipole Moment - 0.012 Molecular Volume - 0.821

Where R^2 = 0.801, SEE = 0.383, SE(ΔG_{CDS}) = 0.064, SE($\Delta G_{lipophilicity}$) = 0.067, SE(Dipole Moment) = 0.048, SE(Molecular Volume) = 0.005, Significance F = 0.013

The binding of FQ and Q drugs to 6-mer single stranded DNA oligonucleotides is correlated with potency IC_{50}.[59] [Noble 2003[59]] The binding (apparent K_D) to ss-DNA for Difloxacin, Enofloxacin, Norfloxacin, Pefloxacin. Ciprofloxacin, 8-Fluoro-Ciprofloxacin, 8-Chloro-Ciprofloxacin, 8-Bromo-Ciprofloxacin, 8-Ethoxy-Ciprofloxacin, Oxalinic Acid, as *anionic* species shows the following relationship

Equation 30

Log App K_D = 0.050 ΔG_{CDS} + 0.034 $\Delta G_{lipophilicity}$ - 0.007 Dipole Moment + **0.013 Molecular Volume** + 4.067

Where R^2 = 0.704, SEE = 0.178, SE(ΔG_{CDS}) = 0.030, SE($\Delta G_{lipophilicity}$) = 0.040, SE(Dipole Moment) = 0.002, SE(Molecular Volume) = 0.022, Significance F = 0.130

Discussion

FQ whole organism activity

It has been shown that the general equation can describe the anti-bacterial (*E. coli*, eq 1) and anti-malarial activity (parasitic protozoa, *Plasmodium falciparum,* chloroquine-sensitive 3D7, eq 2) and (*Plasmodium falciparum,* chloroquine-resistant NF54-R, eq 3). *Plasmodium yoelii yoelii* is also strongly correlated eq 4 and 4a.

The essential difference between the FQ activity against the *Plasmodium falciparum* chloroquine-sensitive 3D7 strain and chloroquine-resistant NF54-R strain is a greater sensitivity to lipophilicity (and lesser sensitivity to desolvation and dipole moment) for the resistant strain. This may imply that hydrogen bonding and polar interactions are relatively reduced in resistant strains when bound to FQs (see discussion below on FQ mechanism).

Ring stage parasitized erythrocytes were used for the 3D7 and NF54-R *Plasmodium falciparum* sudies. Mouse or human hepatocyte cultures were sporozoite inoculations for the *P. yoelii yoelii* studies.[43] [Mahmoudi 2003[43]] Comparison of eqs 2,3 with eq 4 show that the effect of the anti-malarials on the erythrocytes is very different from the effect on the hepatocytes with a large sensitivity on the lipophilicity and dipole moment (and lesser effect on the desolvation) for the hepatocytes. This observation is consistent with the more lipophilic environment in the liver tissue than for the red blood cells where a far more hydrophilic environment exists.

Since the FQs can exist in various neutral, ionic or zwitterionic forms around the physiological pH, the correlations were tested against the various species, focussing on the strength of the correlations and using outlier analysis. It was shown that *E. coli* eq 1 gave a very poor correlation using neutral FQs, but a strong correlation using zwitterionic FQs. Siimilarly the *Plasmodium* correlations found that in eq 2 and 3 Pefloxacin, Sparfloxacin, Levoflocacin and Ofloxacin were far better correlated when treated as zwitterions, whereas the other 16 FQs were treated as neutral species. It appears that the general equation can differentiate amongst the various FQ species statistically, possibly indicating mechanistic differences within a species, and between different microbe families.

The general equation also accurately describes the concentrations of FQs in human seminal and prostatic fluids (and prostatic tissue) ie the prostatic fluid:plasma in treating chronic bacterial prostatis, eq 5. All FQs were treated as the neutral species.

The general equation also accurately describes the in vivo study of the steady state uptake (R_{ss}) of FQs into rat brains and the ratio of brain to blood plasma concentrations (F) when treated as neutral species in eq 6 and 7.

The overall results from eq 1-7 clearly demonstrate the general equation can successfully describe whole organism efficacy of FQs, as it applies to bacterial species, parasitic protozoa, humans and rats.

FQ oral intake, dissolution, gastrointestinal fluid diffusion

The rate determining step for movement of an orally ingested drug into the systematic blood system is the rate of dissolution. Oral Kinetic solubility is the concentration of a compound in solution when an induced precipitate first appears, whereas equilibrium solubility is the concentration of compound in a saturated solution when excess solid is present, and solution and solid are at equilibrium. Kinetic solubility is commonly used in early drug discovery

protocols. Equations 11 and 12 for kinetic solubility are very similar to those for eqs 8,9 and 10 which apply to equilibrium solubility. The best correlations for equilibrium solubility used FQs as the neutral or anionic species (with Ofloxacin as an zwitterion) whereas for kinetic solubility it was FQs in the anionic form which gave the better correlations (with Ofloxacin a zwitterion). Since FQs can exist as charged, neutral or zwitterionic forms at equilibrium at physiological pH, whereas kinetic dissolution requires the change from the solid state (with ionic lattice forces) to solution, these results are consistent with the experimental findings. It is also noted that a much more statistically rigorous analysis of over 70 common drugs also is well described by a similar equation to those in eqs 8-12.[5] [Fong 2016[5]]

FQ human intestinal permeability, renal organic anion transporters, BBB permeability

It is widely considered that only neutral species can passively permeate cell membranes, cationic species may also permeate cell membranes, either by passive permeation or facilitated diffusion, but anionic species are widely considered not to passively permeate cell membranes. However, there are active transporters which can transport charged species across cell membranes of enterocytes, such as the ABC and SLC families.[13] [El-Kattan 2012[13]]

Avdeef[48] and Wang[49] have calculated human jejunal permeability and absorption from experimental Caco-2 data, assuming passive diffusion (transcellular and paracellular) and have also calculated human jejunal permeability rates (passive permeabilities 10^{-4} cm/s). Strong correlations eq 13 and 14 have been found with their permeability data when treating the FQs as neutral species.

The PAMPA intrinsic permeability coefficients for the *uncharged* forms of 4′N-R-norfloxacin, 4′N-R-ciprofloxacin, and 4′N-R-3′-methylciprofloxacin (where R=H, Me, *n*-Pr, *n*-Bu) was strongly correlated in eq 15 and 16. As PAMPA permeation is purely passive permeation (unlike Caco-2 cells which have some active transporter capability), these equation show how large R substituents can affect the permeability. This is particularly shown in eq 16 where the molecular size coefficient is large compared to the other coefficients. This result reflects the use of ΔG_{CDS} rather than $\Delta G_{solvation}$ in eq 16, which is the non-electrostatic contribution of $\Delta G_{solvation}$ and so reflects the non-polar contribution made the R substituents to the PAMPA cell permeation ie the relative contribution $\Delta G_{solvation}$ in eq 15 is larger than the relative contribution from ΔG_{CDS} in eq 16, so emphasising the molecular volume contribution in eq 16.

Eq 17 shows the transport of 9 FQs (mainly as zwitterions, but Ciprofloxacin and Sparfloxacin treated as anions) by renally expressed basolateral SLC22 super family transporters: mOAT (mouse organic anion transporter mOAT3, active in adsorption and excretion of anions). These data are known to include both influx and efflux of renal basolateral cells. The correlation for the active transporter is fairly strong, and the correlation with neutral or all anionic species was very poor.

The steady state in vitro competitive uptake of ^{14}C Grepafloxacin in rat brain capillary endothelial cells in the presence of bicarbonate ions by 7 FQ drugs (as *anions*) is shown in eq 18. The effect on in vitro brain capillary endothelial cell competitive uptake of HSR-903 (Olamufloxacin) by FQs (as neutral species) is shown in eq 19. Eq 18 is a relatively poor

correlation possible reflecting the alkaline conditions and a dynamic equilibria of species. The in vitro data in Eq 19 more closely approximates the in vivo rat data of eq 6 where the pattern of coefficients are similar in relative magnitudes.

FQ binding to plasma proteins

Plasma protein binding PPB affects antibacterial efficacy for all antimicrobial classes, based on its effects on tissue penetration, elimination half-life and the volume of distribution. However PPB also impacts antimicrobial activity by reducing the available fraction of free drug. PPB leads to significant reduction of antimicrobial activity for moxifloxacin and trovafloxacin.[60] [Zeitlinger 2008[60]] Plasma proteins are comprised of ca 55% serum albumin, globulins 38%, fibrogens 7% and the remainder are regulatory proteins. Eqs 20 and 21 show strong correlations with BSA binding using the zwitterionic forms of FQs. The zwitterionic forms of the FQs are dominant at the physiological pH in blood plasma, and it is expected that binding interactions with the BSA would involve significant ionic and hydrogen bonding interactions. We have previously shown that correlations with $\mathbf{\Delta G_{desolvation}}$ are more representative of the largely electrostatic bulk water environment (ε=78.3), whereas the correlations with $\mathbf{\Delta G_{desol,CDS}}$ (which represents the non-polar solvation component of the interaction between the FQ solute and water), is more representative of the interior of the binding pocket of the BSA protein (ε=6-10). Eq 21 is dominated by the non-polar desolvation term, which largely determines binding strength between BSA and the FQs.

FQ serious side effects: hERG cardiac QT prolongation

Hagiwara 2001[54] has assessed the effects of 10 fluoroquinolones on cardiac repolarization by measuring the action potentials recorded from isolated guinea pig right ventricular myocardia tissue under constant conditions, namely, myocardial preparations were electrically stimulated at the fixed pacing frequency of 1 Hz. Sparfloxacin was the most active drug, at 10 μM, prolonged APD_{90} 8.4%, and APD_{50} 9.0%. All other drugs were measured at 100 μM as the APD_{90} values. Blockade of I_{Kr} leads to a delay in the cardiac repolarization and prolongs the APD of myocardia and consequently prolongs the QT interval on the ECG. The APD prolonging potency was sparfloxacin > moxifloxacin = grepafloxacin > gatifloxacin > tosufloxacin = gemifloxacin = ciprofloxacin = trovafloxacin = sitafloxacin = levofloxacin. This I_{Kr} blockade order was the same found for rabbit and canine Purkinje fibers, as shown in Table 1.[61] [Camm 2004[61]] This order closely approximates the order found by Kang 2001[55] from inhibition of hERG channel currents using chinese hamster ovary cells (CHO cells) transfected with cDNA encoding the hERG K_1 channel cloned from a human neuroblastoma cell line. These experimental results agree with clinical practice data which is that Moxifloxacin carries the highest risk of I_{Kr} blockade (noting that Grepafloxacin was withdrawn from sale for causing adverse cardiac effects and that Sparfloxacin was also withdrawn from sale in most countries).

	I_{kr} Blockade (Descending order)	↑ APD^{*}_{90} (Descending order)	↑ QT (ms)	TdP
Sparfloxacin			9–28 (200–800mg)	+ (rabbit/dog)
Grepafloxacin			10	+ (human)
Moxifloxacin			6±26	?
Gatifloxacin		?	2.9±16.5	?
Levofloxacin		–	>60ms (9% of pts)	+ (human)
Ciprofloxacin			?	+ (human)
Ofloxacin		–	?	?

*Canine or rabbit Purkinje fibers.

Table 1. The effect of fluoroquinolones on I_{Kr} blockade, action potential prolongation (ADP_{90}), QT prolongation, and Torsades de Pointes (TdP). (Camm 2004)

Eqs 22 and 23 show reasonably strong correlations for the ADP_{90} data when treating the FQs as neutral species (Tosufloxacin, Gemifloxacin, Sitafloxacin as zwitterions) with eq 23 being the more significant.

Kang 2001[55] has examined the effect of FQs on chinese hamster ovary cells (CHO cells) transfected with cDNA encoding the hERG K_1 channel cloned from a human neuroblastoma cell line. Sparfloxacin was the most potent compound, IC_{50} 18 μM, whereas ofloxacin was the least potent compound, IC_{50} 1420 μM. QT prolongation observed clinically with administration of sparfloxacin and certain other fluoroquinolones showed a relationship with the free plasma levels after therapeutic doses was very similar to those concentrations that inhibit HERG channel current. However, levofloxacin, ciprofloxacin, and ofloxacin inhibition of HERG occurred at concentrations much greater than those observed clinically. It was concluded that clinically relevant hERG channel inhibition is not a class effect of the fluoroquinolone antibacterials but is highly dependent upon specific substitutions within this series of compounds. Eq 24 shows a good correlation with IC_{50} for the CHO cells which can be compared to eq 22 for the APD_{90} data from isolated guinea pig right ventricular myocardia tissue. The equations are similar but the signs of the independent variables are opposite, but the magnitudes are similar.

Eqs 24 and 25 or 26 and 27 where the IC_{50} values are corrected for the peak plasma concentrations of the FQs are very similar except the coefficients are roughly 12 times less sensitive for eq 25 (compared to eq 24) and 11 times less sensitive for eq 27 (compared to eq 26). These equations clearly show that the concentration inside the cells where activity occurs are lowered proportionally to the peak free plasma concentrations of the FQs.

Ryu 2013[62] have examined the binding modes of Levofloxacin and Sparfloxacin with the hERG channel which is a tetrameric protein and binds with a 1:1 stoichiometry. It was concluded that two Tyr652s in the neighbouring subunits and one or two Phe656s in the diagonal subunits contributed to the blockade in the case of both compounds, and Ser624 was also involved. Docking studies suggested that the protonated carboxyl group in the compounds strongly interacts with Phe656 as a π acceptor.

Binding of drugs in the hERG channel has been previously considered to be dominated by: (a) hydrophobicity and or π-stacking interactions with Tyr652 and Phe656 on the S6 helix of

the channel. Multiple aromatic or aromatic groups are critical for high affinity block. (b) flexibility to allow conformational change in the channel, (c) increasing the number of aromatic rings, (d) the presence of a charged amine group can (but not always) increase potency, (e) hydrophilic groups can decrease binding. The inhibitor orients itself along the pore axis, with the lipophilic end facing the opening to the cytosol and the polar tail facing the selectivity filter. One or two hydrophobic moieties interact with Phe656 side chains, probably via π-stacking(Figure 3). Multiple ligand binding sites exist on hERG that are capable of modulating channel activity. [Vanderburg 2012[39], Aronov 2005[61]]

Eqs 22-27 show that FQ binding to the tetrameric protein of the hERG channel is multifaceted, with desolvation of the FQ (and probably of the protein at Ser624) is critical as well as the hydrophobic interactions at Phe656s and Tyr652s, the dipole moment (as an indicator of polar interactions between the FQ and protein) and molecular sizes of the FQs.

A major hurdle when constructing models using literature data has been the large discrepancy observed for hERG IC_{50} values determined in different laboratories. Inter-laboratory variability of greater than 10-fold is not uncommon, even in cases when inhibition was measured using the same cell line.[63] [Aronov 2005[63]] This is generally true when assessing biological activity data for ligand drugs and particularly protein receptors, and using internally consistent data from the same sources has been found to be crucial in defining relationships where mechanistic conclusions are sought.[1-5] [Fong 2015]

FQ anti-bacterial mechanism

The inhibition of gyrase supercoiling activity (IC_{50}) by 12 FQ and Q drugs and the binding of FQ and Q drugs to 6-mer single stranded DNA oligonucleotides (apparent K_D) has been analysed in eqs 28,29 and 30. The correlations with the non-electrostatic CDS values as opposed to the desolvation energy were superior, as shown in equation 29 and 30 as expected since non-electrostatic CDS correlations have been shown to better represent drug-protein binding within the binding pocket. Eqs 29 indicates that the potency of FQ inhibition of gyrase supercoiling activity is primarily dependent on lipophilicity and dipole moment of the FQ, with smaller but significant dependencies on desolvation and molecular volume. Eqs 30 indicates that the potency of FQ binding to ssDNA is primarily dependent on desolvation and lipophilicity, with smaller but significant dependencies on dipole moment and molecular volume.

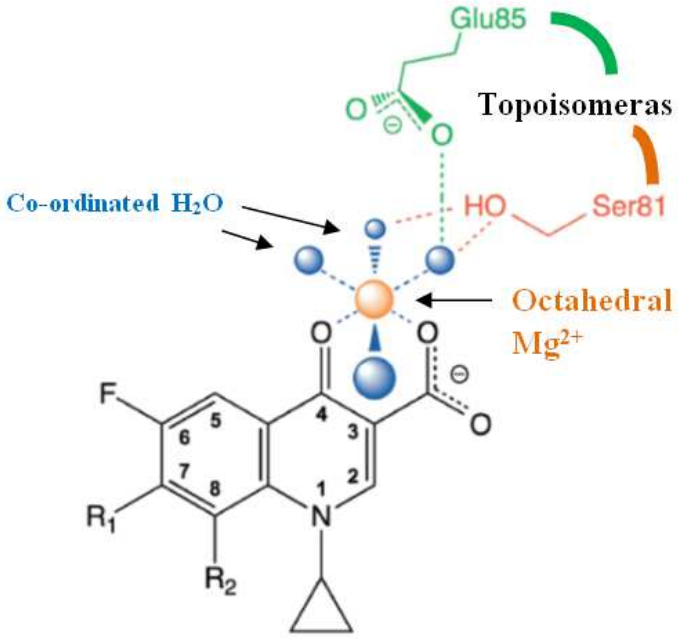


Figure 2. Schematic representation of fluoroquinolone—Mg—Topoisomerase bridging interaction

It has been proposed that one mode of resistance to fluoroquinolones may be associated with the magnesium ion water bridge to the bacterial type IV topoisomerases where the bridge is anchored to the serine and glutamic acid residues (Figure 2). The mechanism of quinolone resistance is suggested to be a result of serine mutations in the topoisomerase which causes partial loss of the Mg-H_2O-topoisomerase bridge.[37] [Aldred 2014[37]] The X-ray structures (PDB 2XKK) show the Moxifloxacin bond lengths C_4=O 1.26A, chelated carboxyl C=O 1.21A, C_4=O—Mg 1.99A, carboxyl C=O—Mg 1.90A. The 4 water molecules octahedrally co-ordinated to Mg were set at 2.07A as per the X-ray structure (2XKK) and the separately characterised $Mg(Ofloxacin)_2$ complex.[64,65] [Wohlkonig 2010[64], Dravensek 2006[65]] The hydrogen bonds between the serine **O**—**H**$_2$O—Mg and glutamic acid carboxylate C=**O**$^{--}$—**H**$_2$O—Mg were arbitrarily varied from 2.60-3.0A and 2.30-2.80A to reflect the weaker hydrogen bond to the serine residue and the ionic bridge to the glutamate carboxylate anion.

The thermochemical analysis of the Moxifloxacin--Mg--(Serine, Glutamate) complex gave **ΔG** values (298K) for the MoxifloxacinMg to Serine,Glutamate interaction in the range of 7.5-9.5 kcal/mol in water. The values are estimates only, as no account was taken of any wider topoisomerase interaction other than direct hydrogen and ionic bonding to the serine and glutamate residues, and the hydrogen bond lengths are not precisely known from the X-ray structure. It is possible to compare these values with the thermodynamically derived complex interaction (using fluorescence emission spectroscopy) between DNA and several quinolones (Norfloxacin, Ciprofloxacin, and Ofloxacin) which gave **ΔG** values between 4.5 and 5.5 kcal/mol.[66] [Lee 2009[66]] It has been shown that the potency of a series of fluoroquinolones is related to the binding to 6-mer single stranded DNA oligonucleotides.[57] [Noble 2003[57]] The presence of the MoxifloxacinMg ion, and the glutamate ion, is expected to significantly increase the interaction with Serine/Glutamate residues of the topoisomerase

compared to bare fluoroquinolones interacting with DNA. These data suggest that the Mg ion may play a significant role in fluoroquinolone activity through a fairly strong interaction with topoisomerase residues.

The physiological bacterial concentration of free Mg^{++} is about 1-2 mM[67] [Snavely 1990[67]], while the intracellular concentration of fluoroquinolone has been calculated to be ~0.1mM.[68][Lecomte 1994[68]] The binding constant for Mg(II) to ciprofloxacin is K_a = $1.30x10^3$ M^{-1} [Lecomte 1994[68]] (the binding constant[69] [Palu 1992[69]] for Mg^{++} to DNA is K_a = $22x10^3$ M^{-1}). It can therefore be readily assumed that fluoroquinolones are complexed with Mg^{++} in the bacterial cell, though the equilibrium constants can vary with different fluoroquinolones and bacteria.

These detailed mechanistic results are consistent with the findings from this study, in particular eqs 28,29 and 30 describing the inhibition of gyrase supercoiling activity and the binding to 6-mer single stranded DNA oligonucleotides. Desolvation of the FQs prior to protein binding is clearly a critical step that would be involved in establishing FQ-Mg-water-Ser,Glu bridging.

It is also noted above (eq 2,3) that the essential difference between the FQ activity against the *Plasmodium falciparum* chloroquine-sensitive 3D7 strain and chloroquine-resistant NF54-R strain is a greater sensitivity to lipophilicity (and lesser sensitivity to desolvation and dipole moment) for the resistant strain. This indicates that hydrogen bonding and polar interactions are relatively reduced in resistant strains when bound to FQs. Similarly desolvation has been shown to be the dominant factor influencing the effect of inhibitors against the cyclin-dependent kinases, but lipophilicity and dipole moment are also significant factors in the resistance shown by MDR proteases to the anti-viral drugs.[1] [Fong 2016[1]]

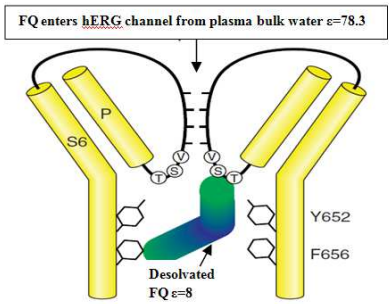


Figure 3. Fluoroquinolone binding in the pore cavity of hERG channels. Solvated fluoroquinolone enters the channel, and partially desolvates prior to binding to channel proteins. Residues involved in fluoroquinolone binding possibly include Thr623, Ser624, and Val625 close to the intracellular entrance to the selectivity filter as well as two aromatic residues Tyr652 and Phe656. Open state only shown, but binding can possibly occur in inactived state. (adapted from Vanderberg 2012[39])

Conclusions

It has been shown that the general equation can accurately describe the activity of FQs against whole organism anti-bacterial and anti-malarial parasites, the steady state uptake into rat brains, and the concentrations of FQs in human seminal and prostatic fluids (and prostatic tissue) when treating chronic bacterial prostatis. *The reason that the general equation can describe whole organism effects is that many of the important individual pharmacological steps that contribute to such whole organism effects are also described by the same general equation.*

Desolvation / solvation effects have been shown to be an important (but usually overlooked) aspect of the most of the pharmacologically processes involved in FQ dissolution, intestinal permeation, systematic circulation, clearance, plasma protein binding, uptake and efflux into bacteria, binding to topoisomerases - DNA, and hERG QT prolongation. Most of these processes involve multi-faceted interactions amongst desolvation, lipophilicity, polar interaction and molecular size of the FQs and the environment, membrane, active anion membrane transporter, protein or hERG transport channel, or binding inhibitory interactions with gyrase supercoiling and 6-mer single stranded DNA oligonucleotides.

Results from the study of sensitive and resistant strains of the malarial Plasmodium parasite show a greater sensitivity to lipophilicity (and lesser sensitivity to desolvation and dipole moment) for the resistant strain. This indicates that hydrogen bonding and polar interactions are relatively reduced in resistant strains when bound to FQs. This conclusion is supported by detailed binding free energies of the FQ-Mg-water-Ser,Glu bridging in type IV topoisomerases.

It is also shown that serious side effects of FQs such as hERG QT prolongation can be predicted for new FQs. Another important conclusion that arises from this study is that the various neutral, ionic and zwitterionic species which can exist around the physiological pH can be differentially involved in the various processes.

The general equation or its modified form is a useful guide to drug discovery and design, particularly the allowing examination of the various species of a potential drug that may predominate at different pH levels, or by making changes to the molecular structure to predict binding or transport properties.

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Table 2. Solvation energies, lipophilicities, dipole moments and molecular volumes for fluoroquinolones, quinolones and related compounds

Fluoroquinolones Quinolones	**$\Delta G_{solvation}$** kcal/mol (CDS) Water	**$\Delta G_{lipophilicity}$** kcal/mol n-Octane	**Dipole Moment** D Water	**Molecular Volume** cm^3/mol Water
Moxifloxacin	-19.5 (8.7)	-15.4	12.2	262
Moxifloxacin Zw	-109.7 (11.2)	-44.0	62.9	268
Moxifloxacin Ion	-90.1 (8.6)	-38.2	37.1	305
Lomefloxacin	-16.2 (4.1)	-10.3	8.2	210
Lomefloxacin Zw	-103.0 (6.4)	-39.4	56.8	226
Lomefloxacin Ion	-82.3 (3.8)	-31.8	32.4	233
Enoxacin	-21.1 (5.4)	-14.3	10.3	225
Enoxacin Zw	-109.2 (8.2)	-42.5	56.4	197
Enoxacin Ion	-91.7 (5.2)	-37	32.3	212
Norfloxacin	-20.5 (1.8)	-11.4	10.8	230
Norfloxaci Ion	-89.6 (1.5)	-33.6	33.3	189
Norfloxacin Zw	-110.2 (4.6)	-41.4	57.9	215
Sparfloxacin	-22.5 (8.0)	-14.8	13.7	281
Sparfloxacin Zw	-109 (10.2)	-43.2	61	255
Sparfloxacin Ion	-108.5 (7.9)	-42.6	58	238
Ofloxacin	-20 (2.8)	-12.1	9.9	224
Ofloxacin Zw	-102.7 (6.45)	-39.7	57.8	224
Ofloxacin Ion	-87.8 (2.5)	-33.9	34.3	275

Grepafloxacin	-19.6 (7.0)	-14.6	12.8	253
Grepafloxacin Zw	-108.2 (9.6)	-43.2	59	201
Grepafloxacin Ion	- 90.8 (6.9)	-37.7	35.2	270
Ciprofloxacin	-20.6 (1.9)	-12.4	10.6	226
Ciprofloxacin Zw	-110.0 (4.6)	-42.5	57.7	184
Ciprofloxacin Ion	-89.6 (1.6)	-34.7	32.9	245
Gatifloxacin	-19.8 (8.6)	-14.2	13.5	251
Gatifloxacin ZW	-108.2 (11.25)	-42.9	59.4	219
Gatifloxacin Ion	-91.1 (8.5)	-37.4	36.6	233
Danofloxacin	-18.9 (1.7)	-12.7	7.1	205
Danofloxacin Ion	-85 (1.45)	-34.2	31.4	218
Sarafloxacin	-18.5 (4.4)	-13	9.5	248
Sarafloxacin ZW	-106.8 (7.1)	-42.8	57.1	257
Sarafloxacin Ion	-86 (4.7)	-34.7	33.3	259
Pefloxacin	-19.9 (6.4)	-13.8	13.8	249
Pefloxacin Zw	-109 (9.5)	-43.2	61	255
Pefloxacin Ion	-91.2 (6.3)	-36.9	36.9	210
Flumequine	-12.5 (5.6)	-9.8	10.6	185
Flumequine Ion	-82.6 (5.3)	-32.4	27.4	196
Fleroxacin	-19 (7.6)	-13.1	10.9	225
Fleroxacin Zw	-104.9 (10.6)	-41.8	59.1	223
Fleroxacin Ion	-86.4 (7.5)	-35	34.9	227
Levofloxacin	-22.5 (6.8)	-14.8	13.7	281
Levofloxacin Zw	-108.5 (10.2)	-42.6	58	238
Levofloxacin Ion	-94.9 (6.7)	-38.2	35.8	259
Prulifloxacin	-28.9 (10.6)	-19	18.3	284
Prulifloxacin Zw	-117.3 (13.4)	-46.0	62.1	283
Prulifloxacin Ion	-96.6 (10.5)	-40.5	45.4	308
Chlorequine	-10.15 (2.45)	-11.5	5.93	356
Clinifloxacin	-18.7 (8.0)	-13.9	12.1	254
Rufloxacin	-20.4 (2.2)	-12.9	9.3	233
Trovafloxacin	-21.8 (11.65)	-14.35	20.3	249
Tosufloxacin	-18.6 (9.1)	-14.5	10.6	251
Tosufloxacin Zw	-111.5 (10.7)	-44.3	59	250
Gemifloxacin	-23.5 (8.9)	-16	9.9	220
Gemifloxacin Zw	-111.7 (10.3)	-45.5	64.9	250
Sitafloxacin	-18.9 (8.1)	-14.5	10.4	254
Sitafloxacin Zw	-111.0 (9.3)	-46.3	60.2	239
Enrofloxacin	- 21.1 (1.88)	-13.2	10.3	224
Enrofloxacin Ion	-88.4 (1.9)	-35.4	35.6	211
Difloxacin	-17.8 (4.3)	-13.2	9.2	266
Difloxacin Ion	-85.2 (3.97)	-34.8	34.3	262
Pipemidic Acid	-21.4 (-0.25)	-13	7.4	241
Nalidixic Acid	-15.9 (4.9)	-9.7	7.1	176
Piromidic Acid	-18.2 (1.9)	-13.6	8.6	212
Olamfloxacin	-19.1 (8.8)	-14.4	10.2	270
Olamfloxacin Ion	-90.8 (8.7)	-37.5	34.8	267
Orbifloxacin	-19.2 (8.1)	-13.7	11.2	254
Orbifloxacin Zw	-107.6 (10.2)	-41.8	59.9	257