QUINOLONES FOR APPLICATIONS IN MEDICINAL CHEMISTRY: SYNTHESIS AND STRUCTURE

DOI: http://dx.medra.org/10.17374/targets.2019.22.260

Pedro Horta[a], Alina Secrieru[a,b], Andy Coninckx[a], Maria L. S. Cristiano[a,b]

[a]*Center of Marine Sciences, CCMAR, Gambelas Campus, University of Algarve, UAlg, Portugal*

[b]*Department of Chemistry and Pharmacy, Faculty of Sciences and Technology, FCT, Gambelas Campus, University of Algarve, UAlg, Portugal*

(e-mail: mcristi@ualg.pt)

Abstract. *Quinolones (oxo-quinolines) are a versatile sub-group within the quinoline family with potential applications in major fields, namely in medicine. Several quinolone-derived compounds are used as drugs, targeting a variety of diseases, e.g. bacterial infections, cancer, hepatitis, HIV, herpes, fungal infections, immunodepression, neurodegenerative diseases, tuberculosis or malaria. In addition to these and other relevant applications, the chemical properties of quinolones, namely their structure and reactivity, also attract attention, stimulating a perennial interest in this class for several decades. Given the wealth of applications of quinolones, the availability of mild, high yielding, selective and versatile synthetic routes for their preparation is of upmost relevance. However, the few available synthetic routes to the quinolone sub-class are not so reliable, for instance from the viewpoints of selectivity and versatility, often leading to poor yields of the isolated products. We recently demonstrated that these problems are linked to structural features, such as the possibility of isomerism or tautomerism.*

Contents

1. Introduction

Quinolines are a group of compounds derived from a bicyclic aromatic fused six-membered heterocyclic nucleus containing one to four nitrogen atoms.[1] The incorporation of a carbonyl group at any position of the quinoline core yields oxo-quinolines/quinolones.[1] The structures of 2-oxo-quinoline (also known as 2-oxo-1,2-dihydroquinoline or as 2(1*H*)-quinolone) and 4-oxo-quinoline (also known as 4-oxo-1,4-dihydroquinoline or as 4(1*H*)-quinolone) are represented in Figure 1. The present chapter will focus on quinolones, with special emphasis on 2-oxo- and 4-oxo-quinolones.

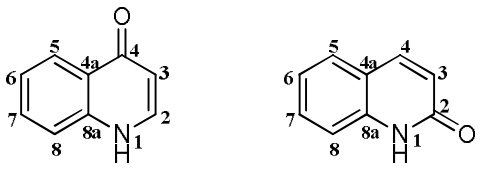


Figure 1. Structural representation of 4(*1H*)-quinolone and 2(1*H*)-quinolone.

The versatility of the quinolone chemotype has attracted intense research for several decades among chemists and medicinal chemists, owing to their vast distribution in nature and immense therapeutic potential, and the interest in this class of compounds does not appear to fade.[2] Quinolones have emerged as drugs and drug candidates, targeting a variety of human diseases and disorders, including infectious diseases, such as malaria and other parasitic infections,[3-6] bacterial infections and tuberculosis,[7-9] fungal infections[10] and viral infections[11-13] such as hepatitis,[11] HIV[12] and herpes.[13] In addition, several studies demonstrated the potential of quinolones for the treatment and/or control of acute and chronic diseases, including pain, ischemia, immunomodulation,[14] inflammation[15] and cancer.[16] This chapter reviews the uses of oxo-quinolines in medicinal chemistry and the synthetic methodologies available for their preparation, emphasizing some structural features of the class and their impact on reactivity.

2. Quinolones in medicinal chemistry

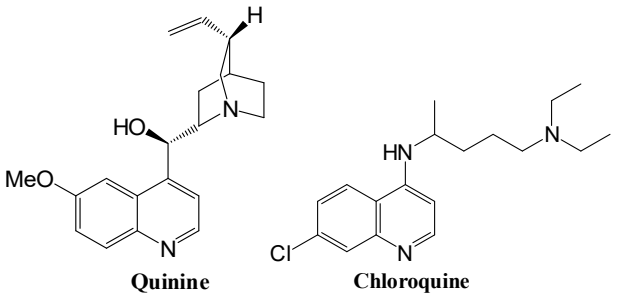
2.1. Quinolones as antimalarial agents

Malaria is a deadly infectious disease that has affected mankind for several millennia. It is caused by a protozoan parasite of the *Plasmodium* genus and transmission of the parasite occurs *via* the bite of an infected female *Anopheles* mosquito (vector).[17] The control of malaria has long been, and remains, a priority, especially because of the fast development of parasites resistant to almost all available antimalarial drugs.

The use of quinoline derivatives as antimalarial drugs in Europe goes back to the beginning of the 17th century, when Jesuit priests returning from Peru introduced an extract from *Cinchona*, a tree originally found in the high hills of South America, as a treatment for malaria.[18] The bark of this tree was later found to be a mixture of about 35 alkaloids, from which the antimalarial drug quinine (Figure 2) would be isolated.[19,20] Initially, the therapeutic properties of the *Cinchona* bark were inconsistent, due to the difficulty in distinguishing *Cinchona* from other trees and also to the variability in quinine content in different *Cinchona* species. Nevertheless, its popularity was such that in 18th century several species of *Cinchona* trees were becoming extinct. In the early 19th century quinine was finally isolated and established as the active pharmaceutical ingredient, replacing the crude bark in malaria chemotherapy. Investigations on the mode of action of quinine yielded evidence that quinoline-like compounds (*Cinchona* alkaloids, their derivatives and synthetics analogues) interfere with *haem* detoxification, by binding to the porphyrin ring system of haem or by raising intravesicular pH, inhibiting polymerization of toxic *haem* towards the non-toxic physiologic polymer.[21,22] The relevance of quinine boosted research towards its synthesis for decades. A synthetic access

to quinine was proposed by Woodward and co-workers in 1944 and research efforts by several groups yielded other quinoline-derived antimalarial drugs.

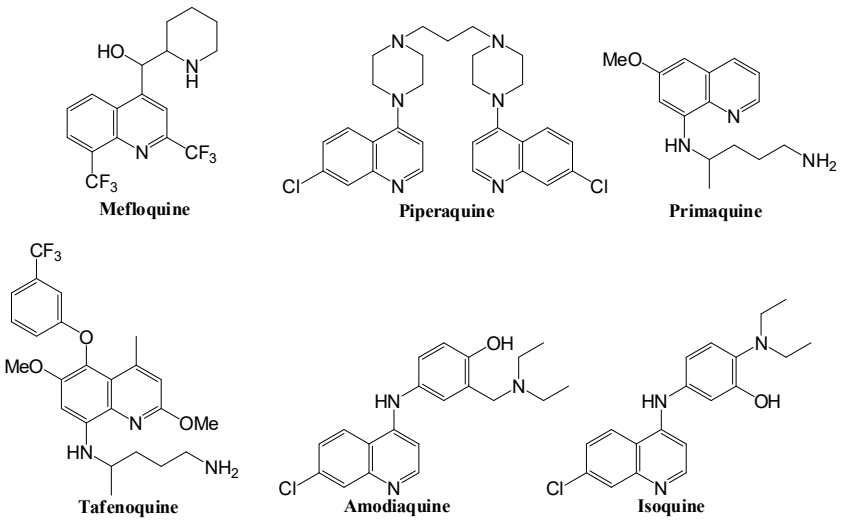
The discovery of methylene blue by the end of the 19th century led to the synthesis of 8-aminoquinolines (pamaquine, mepacrine) and 4-aminoquinolines with potent toxicity against *Plasmodium spp.*[18-20] **Chloroquine** (Figure 2), the first antimalarially active 4-aminoquinoline obtained from synthesis, was reported in 1934. This drug demonstrated to be highly effective against *Plasmodium* parasites, well-tolerated by humans (less toxic and more effective than quinine) and, in addition, was cheaper than other contemporary antimalarial drugs.[22,23] However, resistance of erythrocytic asexual forms of *P. falciparum* to chloroquine has been described shortly after its discovery and is currently common in all endemic areas around the world. Despite these limitations, chloroquine is still used for prophylaxis and treatment of malaria in the few African regions where *P. falciparum* remains sensitive to this drug.[21-24]



Quinine **Chloroquine**

Figure 2. Structural representation of quinine and chloroquine.

The efforts to the discovery of new antimalarials effective against chloroquine-resistant strains of *Plasmodium* spp. resulted in the development of various quinoline derived drugs, represented in Figure 3 (mefloquine, piperaquine, primaquine, tafenoquine, amodiaquine and isoquine). However, some of these new drugs evidenced limitations, mainly related to toxicity and cost, and were soon overshadowed through the development of drug resistance by the parasite.[21-23] Nowadays, quinine remains an alternative antimalarial drug to treat infections caused by parasite strains resistant to other drugs (*e.g.* chloroquine), although side effects related to its use are known.



Mefloquine **Piperaquine** **Primaquine**

Tafenoquine **Amodiaquine** **Isoquine**

Figure 3. Structural representation of quinoline-based antimalarials with historical and/or current relevance.

While the growing emergence of resistance to antiplasmodial drugs continuously demands the development of new and effective weapons to fight malaria, recent advances suggest that previously abandoned lead molecules may be reexamined to generate more robust and potent antimalarial drugs. A notable example is the development of new endochin analogues (or endochin like quinolones-ELQ) with improved therapeutic properties compared to the parent compound. **Endochin** (Figure 4), a 2,3,7-substituted 4-oxo-quinoline developed by Bayer, established the antiplasmodial activity of quinolones in 1948[25] but proved ineffective in mammal models due to its rapid metabolism to inactive metabolites by cytochrome P450 enzymes.[25-29]

Inspired by the structure and properties of endochin, Ryley *et al.* developed a number of 4-oxo-quinoline 3-esters in 1970 and noted that some *e.g.* **ICI 56780** and **ICI 60128** (Figure 4) were very active against malaria when tested in rodent and monkey models, both as prophylaxis and treatment. These compounds appeared to be effective against all stages of the parasite, including chloroquine resistant strains, with a potency of up to 50 times that of chloroquine, but it was noted that the parasite developed fast resistance to the drugs, in rodent models.[30] However the validation of the parasite's mitochondrial electron transport chain (mtETC) as an important therapeutic target, along with the discovery of atovaquone as a potent inhibitor of the cytochrome bc_1 protein complex, kept the quinolone core as model for the synthesis of new potential antimalarial compounds, now targeting specifically the Q_o site of *P. falciparum* bc_1 complex of the parasite (mtETC).[6]

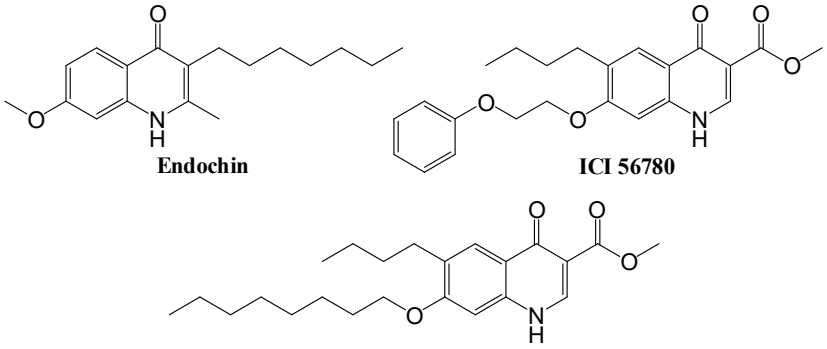


Figure 4. Structural representation of quinolone-based antimalarials; endochin and quinolone 3-esters.

Malaria parasites have retained the ability to generate a large electrochemical potential across their mitochondrial membrane. Therefore, mtETC is critical for parasite survival and, as the inhibition of respiratory enzymes has severe consequences, drugs targeting these enzymes could be efficient tools for pathogen control.[31-33]

However, there are barriers to development of novel inhibitors based on the quinolone core, such as poor solubility, chemical instability and tautomerism,[34-36] the possibility of cardiotoxicity[37] and some mutations associated to atovaquone resistance and cross-resistance to other Q_o inhibitors.[32]

For example, Riscoe and Winter found in 1970 that by extending the 3-heptyl chain of endochin to an undecyl (11C) chain and terminating with a trifluoromethyl moiety (synthesis of **ELQ-103**, Figure 5), the *in vitro* activity against *P. falciparum* chloroquine resistant parasite strains was doubled (IC_{50} 1.4nM *vs* 2.8nM). Additionally, the cross-resistance for atovaquone resistant parasite strains was significantly diminished (IC_{50} 4.7nM *vs* 17.4nM for endochin). However, the solubility and *in vivo* activity of this compound was still very poor.[27]

In 2011, in an effort to increase solubility and reduce metabolic instability, the same research group optimized the structure of **ELQ-103** by changing the substitution pattern of the quinolone ring. From a library with several **ELQs**, **ELQ-121** (Figure 5) was established as a new lead, exhibiting a potency 10-fold higher than **ELQ-103** and enhanced metabolic stability. However, **ELQ-121** exhibited increased cross-resistance with atovaquone, when compared to **ELQ-103**. The problem of solubility was

not circumvented by this drug, but its prodrug (with a poly(ethylene) glycol group linked to the oxygen of the quinolone core) **ELQ-125** (Figure 5) demonstrated a better solubility profile.[38]

Cross *et al.* designed and synthesized several endochin-type quinolones to better understand the structure-activity relationship (SAR) profiles against *P. falciparum*. Some important aspects about SAR were established: the carbonyl at position 4 and the lack of substitution at N-1 are key factors; a halogen substituent (chlorine) at C-6 and/or a methoxy group at position 7 enhance activity and reduce cross-resistance; shortening the 3-alkyl chain length increases the solubility but results in a substantial reduction in parasite selectivity (an alkyl chain with at least 7 carbons at C-3 is preferred); and 3-aryl substituted analogues exhibit a drop in potency, but less severe, while both solubility and metabolic stability are much improved. The most potent analogue from the library studied by Cross *et al*. is Compound55 Manetsch, represented in Figure 5.[39]

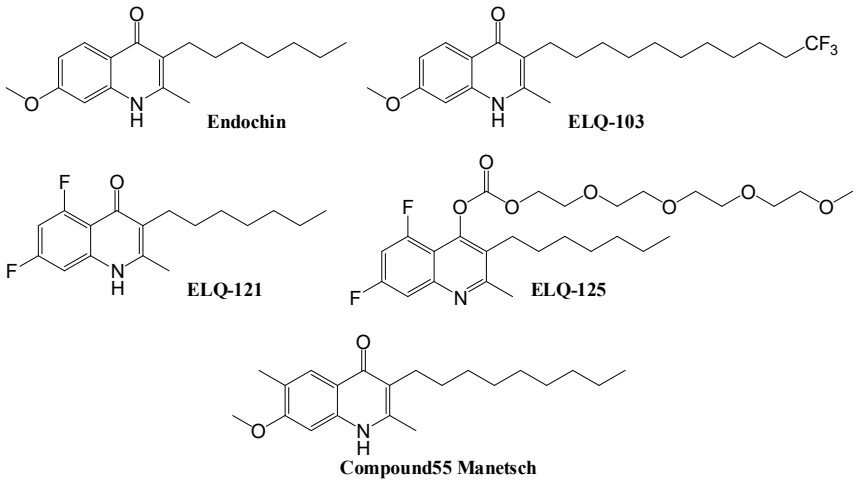


Figure 5. Structural representation of endochin and of **ELQs**, studied by Riscoe *et al.* and Cross *et al.*

More recently, in 2013, Manetsch and Riscoe studied analogues containing a diarylether substituent at C-3 and, from them, the *P. falciparum* bc_1 complex inhibitor **ELQ-300** (Figure 6) was selected as a preclinical candidate (IC_{50} around 1.8nM against chloroquine resistant strains). Considering the side chain of the previously described pyridone (**GW844520**) and the endochin structure, **ELQ-271** (Figure 6) was firstly synthesized and, following the introduction of a methoxy substituent at C-7 and a chlorine substituent at C-6, originated **ELQ-300**. In preclinical studies with mice, **ELQ-300** was found to be highly active against *P. falciparum* and *P. vivax* through all life cycle stages that play a role in the transmission of malaria, and to exhibit good oral bioavailability. **ELQ-300** was also shown to have no effect on intracellular ATP levels in two different mammalian cell lines (no toxicity) and no cross-resistance with atovaquone was observed. Compound **P4Q-391** (Figure 6), containing a fluorine substituent in the diaryl ether side chain, has also demonstrated a promising profile (IC_{50} of around 7.6nM against chloroquine resistant strains). However, **ELQ-300** was shown to be more potent and selective.[40]

In parallel, Da Cruz *et al.* and Cowley *et al*. have investigated a range of quinolone 3-esters as *P. falciparum* bc_1 complex inhibitors. From the libraries investigated, Da Cruz *et al.* have selected decoquinate (Figure 7) as the most potent compound against the liver stages (IC_{50} around 2.6nM),[41] while Cowley *et al*. have proposed **RCQ** (Figure 7) from SAR studies, a highly potent antimalarial exhibiting an IC_{50} value of 0.46nM.[6]

Some quinolone derivatives are also known as potential inhibitors of *Pf*NDH2 in the respiratory chain of *P. falciparum*. Hydroxy-2-dodecyl-4-(1*H*)-quinolone (**HDQ**), represented in Figure 8, exhibits inhibitory activity both towards *Pf*NDH2 and the bc_1 complex and, as such, was used as a starting point

for drug discovery, leading to libraries with several new quinolone analogues bearing promising antimalarial activities. This multi-target inhibition confers a benefit over the single-target inhibition, due to delay in selection for drug resistance.[5]

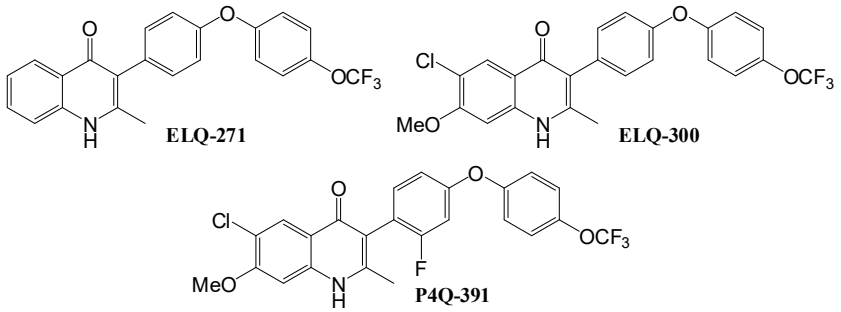


Figure 6. Structural representation of **ELQ-300** (a preclinical candidate), its precursor **ELQ-271** and the analogue **P4Q-391**.

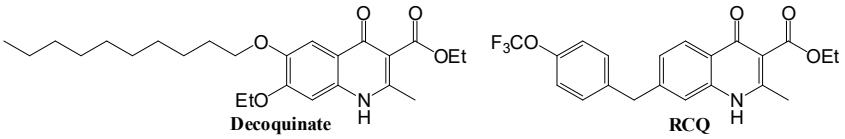


Figure 7. Structural representation of decoquinate and **RCQ**, proposed by Da Cruz *et al.* and Cowley *et al.*, respectively.

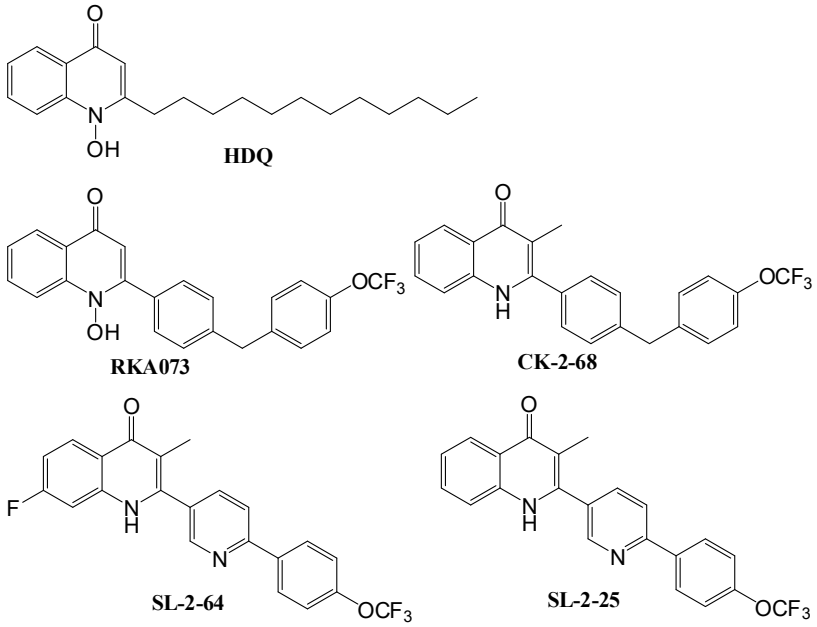


Figure 8. Structural representation of **HDQ** and of other quinolones substituted at positions 2 and/or 3, studied by Pidathala *et al.* and Leung *et al.*.

The metabolic liabilities evidenced by **HDQ** led to additional structural alterations. Pidathala *et al.* changed the HDQ-side chain at position 2 to a biaryl or phenoxyl biaryl *e.g.* **RKA073** (Figure 8). After conversion of N-OH to N-H and introduction of 7-Cl and 3-methyl substituents, **CK-2-68** (Figure 8) was selected as a lead for further development, showing an activity *in vivo* of 31nM against *P. falciparum* (compared with 263nM for **RKA073**).[3]

Leung *et al.* introduced a heterocyclic substituent (pyridyl group) into the quinolone side chain to improve solubility, leading to the analogs **SL-2-64** and **SL-2-25** (Figure 8) that evidenced IC50 values of 75nM and 54nM, respectively, against whole-cell *P. falciparum*.[4] A loss in *Pf*NDH2 activity was observed, when moving from 2-aryl to 3-aryl substituted analogues.[3,4]

2.2. Quinolones as antibacterial agents

The use of quinolones as antibacterial agents (a well-established class of antibiotics) began in 1963, with the discovery of nalidixic acid (Figure 9), a 1,8-naphthyridone 3-acid, during the synthesis of the antimalarial agent chloroquine.[42] **Nalidixic acid**, the first synthetic quinolone-based antibacterial agent, was used against some enteric bacteria in the treatment of urinary tract infections. It is a *first-generation quinolone* presenting favorable structural features, such as the ethyl group at N-1 position, and good Gram-negative activity. However, nalidixic acid never became a useful agent to treat systemic infections, due to its narrow spectrum of action, poor tissue penetrability, rapid emergence of bacterial resistance and frequent adverse effects in the central nervous system.[1,8,43]

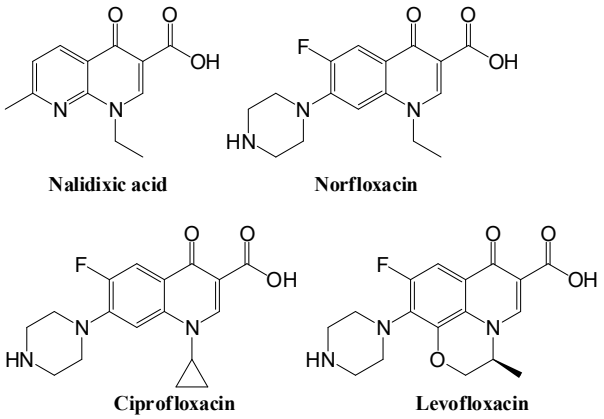


Figure 9. Structural representation of quinolones used as antibacterial agents.

The mode of action of quinolone antibiotics has been investigated and is thought to involve inhibition of bacterial topoisomerases II (DNA gyrase) and IV, responsible for the transient cleavage of single- and double-stranded DNA molecules, an essential step for replication, transcription, recombination and condensed DNA remodeling. Topoisomerases modulate the supercoiling of DNA to enable proper function and interaction with proteins during the replication process. Any interruption in one or more steps of this process causes rapid bacterial cell death.[44,45]

The discovery of nalidixic acid led to the synthesis of a wide variety of broad-spectrum quinolone derivatives and provided the chemical foundations upon which modifications to improve the pharmacologic profile and limit adverse effects were built. More than 10.000 analogues of nalidixic acid were studied and/or synthesized and, after 20 years, only one analogue was approved for clinical use, the first fluoroquinolone antibiotic.[1,46]

Initially, three main features on the quinolone scaffold were associated with the best antibacterial quinolone activities, which were present in nalidixic acid, the first molecule used for this purpose: an ethyl group at N-1, the hydrogen atom at C-2, a carboxylic acid group at C-3 and a carbonyl group at C-4.[47] Modifications at any of the other positions could result in better potency and a broader spectrum of activity.

Substitutions at C-6 and C-7 led to enhanced activity, particularly with a fluorine atom at C-6, as well as additional medium sized, four- to six-membered ring structures containing a N atom at C-7, which allow for an even wider range of action. C-6 fluorine substituted quinolones are known as fluoroquinolones and account for the majority of quinolone-type molecules used clinically against bacterial-borne diseases.[45,47,48]

Fluoroquinolones (Figure 9) present increased activity against both Gram-negative and Gram-positive organisms. The fluorine atom expands the spectrum of action and is also responsible for improving the penetration of quinolones into bacterial cells. There are other aspects in the structure of fluoroquinolones that have direct impact on antibacterial activity: the 3-carboxylate and 4-carbonyl groups were found to mediate the binding to DNA gyrase and the transport across the bacterial membrane, while alterations at N-1, C-5 as well as at position 7 and 8 of the quinolone core were found to affect the potency, spectrum of activity, pharmacokinetics and safety profile.[1]

Norfloxacin, ciprofloxacin (second-generation quinolones) and levofloxacin (third-generation quinolones) are examples of fluoroquinolone antibiotics with a piperazine ring at position 7 (Figure 9), a structural feature that appears to be essential for enhancing potency, spectrum of action and pharmacokinetic profile. However, the comparatively lower potency against some Gram-positive bacteria, particularly *Staphylococcus aureus* and *Streptococcus pneumoniae*, and the remaining adverse effects of this class have prompted further research towards a more extensive utility of fluoroquinolones.[7,8]

Norfloxacin, the first fluoroquinolone (approved in 1986), has poor tissue distribution, which limited its application to the treatment of urinary tract infections. Over the following decades several compounds have derived from this quinolone lead, with the replacement of the substituent at N-1 by a cyclopropyl group leding to ciprofloxacin (approved in 1987), an antibiotic with a better clinical success. Ciprofloxacin presents an excellent systemic activity upon oral administration and is one of the most frequently prescribed antibiotics.[7]

Levofloxacin, a third-generation quinolone, was approved in 1996. It is the *levo* isomer of the racemate ofloxacin (another quinolone-based antimicrobial agent). This generation exhibits wider structural diversity (*e.g.* heteroaromatic ring between N-1 and C-8 for levofloxacin; and methylation of the piperazine ring at position 7 for other analogues not presented in this work) that results in novelties regarding biological properties. The third-generation quinolones preserve excellent potency against Gram-negative bacteria while showing an increased activity towards Gram-positive bacteria, compared to ciprofloxacin.[7,8]

Moxifloxacin and **Gatifloxacin** (Figure 10) are two of the newer fluoroquinolones, bearing a methoxy group at position 8 that favors interaction with bacterial topoisomerases, improving their overall activity.[49]

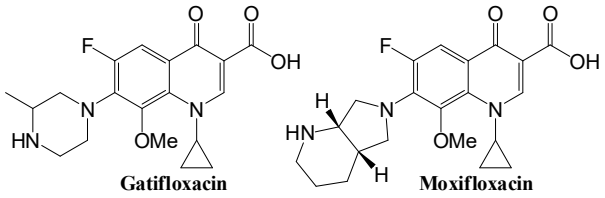


Figure 10. Structural representation of the quinolone-based drugs **Gatifloxacin** and **Moxifloxacin**, used as antibacterial agents.

Besides their potency and broad spectrum of activity, the newer fluoroquinolones present several other advantages, namely in their pharmacokinetic properties. In comparison to the older ones, these have higher bioavailability, lower protein binding and longer elimination half-lives, allowing a once-daily dosing and increased patient compliance. In addition to the previous, recent evidence suggests that their ability to act on both DNA gyrase and topoisomerase IV decreases the rate of chromosomal mutations that lead to resistance.[44,49] The pharmacologic properties of fluoroquinolones justify their extensive use as second line therapeutics, not only in urinary tract infections but also for the treatment of certain sexually transmitted diseases, namely prostatitis, gastrointestinal infections, nosocomial acquired pulmonary infections, pseudomonas infections and osteomyelitis.[7]

As pointed above, the structural relationship of the quinolone core with some antimalarial drugs (for example atovaquone) can open new perspectives for fluoroquinolones in malaria chemotherapy. Quite interesting, during the 1980s and 1990s, fluoroquinolones antibiotics, such as ciprofloxacin, were shown to have antimalarial properties, and the possibility of replacing the failing aminoquinolines by fluoroquinolones was considered, despite the lower potency of fluoroquinolones. However, the advent of more promising antimalarial drugs, like artemisinin and derivatives, devalued the use of fluoroquinolones in malaria.[50-52]

2.3. Quinolones as antimycobacterial agents

Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (MTB) and, most commonly, affects the lungs, causing cough (sometimes with sputum or blood), chest pain, weakness, weight loss, fever and night sweats.[53] Despite the available treatments TB treatment remains a global health problem, since research and development regarding this disease has not evolved for many years until recently and the pathogenic agent has evidenced an increased prevalence of multi and extensive drug-resistant strains (MDR and XDR, respectively).[54,55] The resistance phenomenon may result, mostly, from the misuse of current antituberculosis chemotherapeutic regimens, which involve combinations of, at least, three different drugs and therapeutic regimens of six months or longer, with the possibility of some characteristic side effects.[9,56]

Over the past years, several subclasses of 4-oxo-quinoline derivatives, incorporating various substituents in the structure of the quinoline core, have demonstrated diverse bioactivities, including high activity against bacterial pathogens. Particularly, gatifloxacin and moxifloxacin (Figure 10) are under evaluation in this field, as substitutes for isoniazid and as a possibility of shortening the overall regimen duration, providing greater safety and tolerability profiles in TB treatment.[54,55,57,58]

In addition to the preexisting quinolone drugs under investigation, several other quinolones are being designed for their potential as antituberculosis agents. Some analogues incorporating variations on the 4-quinolone core of the fluoroquinolone drugs resulted in a series of different analogues with good activity.[59]

Some authors suggest that the fluoroquinolone core with a methoxy group at C-8 (moxifloxacin and gatifloxacin derivatives) exhibit improved activity, with variations mainly at C-5 (for example with introduction of a nitro group) and at C-7, with bulky and highly lipophilic groups containing secondary amines, subsequently improving the penetration of these compounds into mycobacterial cells. For example, Senthilkumar *et al.* reported the synthesis of a new series of fluoroquinolones and found compound **1** (Figure 11) as the most active compound, with MIC of 0.16 and 0.33 mM against MTB and MDR-TB, respectively (isoniazide: MIC of 0.36 and 45.57, for MTB and MDR-TB, respectively).[60]

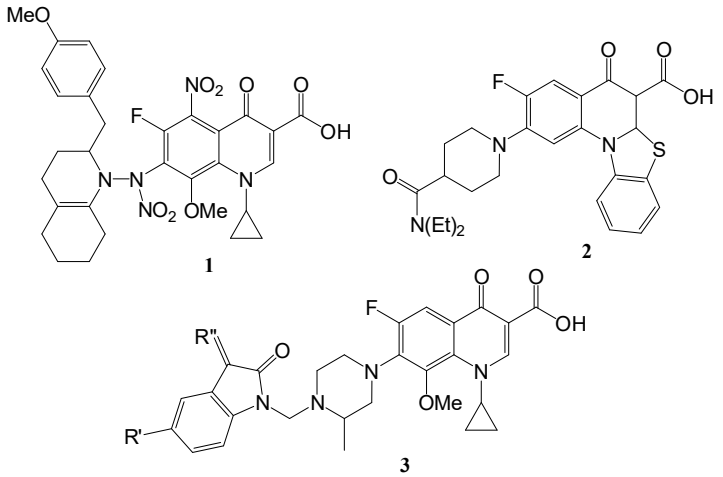


Figure 11. Structural representation of fluoroquinolones with potential antimycobacterial activity, studied by Senthilkumar *et al.*, Dinakaran *et al*. and Sriram *et al.*.

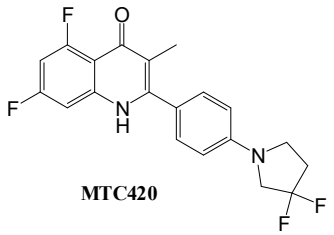
Dinakaran *et al*. studied the antimycobacterial activity profile of various 2-(substituted)-6-fluoro/nitro 4-oxo-quinoline derivatives from which compounds with moderate to promising activities were selected, with compound **2** (Figure 11) showing to be 2 and 570 times more potent than standard isoniazide, against MTB and MDR-TB, respectively (MIC of 0.18 and 0.08 mM against MTB and MDR-TB, respectively). Regarding the effect of a fluorine or a nitro-substituent at C-6, fluoro substituted inhibitors were found to be more active.[61]

In continuation of previous works, Sriram *et al.* evaluated the antimycobacterial activity profile of 7-substituted gatifloxacin derivatives and found that selected compounds within scaffold **3** (Figure 11) displayed good antimycobacterial activities, non-toxicity and good selectivity. Furthermore, it was suggested that increasing the lipophilicity of the side chain at C-7 position improved the antimycobacterial activity.[62]

Besides the presence of bulky lipophilic groups at C-7 and the introduction of alkyl moieties at N-1, other studies proposed that maintaining a hydrogen atom at C-8 improves antimycobacterial activity by the metal chelating property of the 8-hydroxiquinoline moiety.[63] Other variations on the quinolone core have also been proposed, namely alterations of the carbonyl group to positions 2 and 5 and introduction of bulky substituents at C-3 and C-2, respectively, and even fusion of the quinolone scaffold with a triazole moiety.[57]

Recently, based on previous studies of selected quinolones, regarding their antimalarial activity (targeting mitochondrial electron transport chain) and the SAR studies for potential antimycobacterial drugs, Hong *et al.* reported on the evaluation of novel compounds with multiple mechanisms of action.[64] For example, quinolones have demonstrated a dual mechanism of action, targeting two plasmodial respiratory enzymes (*Pf*NDH2 and cytochrome bc_1) and selected quinolones targeting components of the MTB respiratory chain have been shown to be effective, *in vitro* and *in vivo*, against *Mycobacterium tuberculosis* and multidrug-resistant MTB.[5,65] Additionally, as described above, quinolone derivatives may target topoisomerases II (DNA gyrase) and IV.[66]

Hong *et al.* performed a medicinal chemistry SAR study and demonstrated acceptable antituberculosis activity of **MTC420** (Figure 12) against all drug sensitive and multidrug resistant strains of MTB tested (MTB IC50=525 nM, MTB Wayne IC50=76 nM, and MDR MTB patient isolates IC50=140 nM) and favorable pharmacokinetic and toxicological profiles. They established that the most favorable group at C-5 and C-7 is fluorine (5,7-fluoro-4-oxo-quinolines) and, concomitantly, by testing the absence of a methyl group at C-3 proved that the activity is lost without this group.[64]



MTC420

Figure 12. Structural representation of **MTC420**, studied by Hong *et al.*.

2.4. Quinolones as anticancer agents

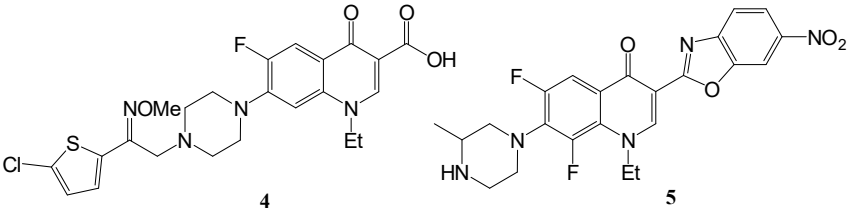
Cancer defines a group of diseases characterized by rapid and abnormal division of cells, which can occur in any part of the body and invade surrounding tissues or even spread throughout the body after reaching the bloodstream. When this aberrant cell division originates a mass of tissue, that tissue is called a solid tumor.[67]

DNA topoisomerases play a key role in cancer since they regulate cell division through modulation of DNA supercoiling process during replication. Quinolones are known for a high inhibitory capacity towards eukaryotic topoisomerases, thus emerging as the potential leads for the treatment and control of solid tumors.[68]

Based on the preexisting fluoroquinolones, which showed activity on human topoisomerase II while used for their antibacterial effect, Foroumadi *et al.* evaluated the introduction of *N*-substituted piperazinyl groups at C-7 position of the ciprofloxacin and norfloxacin core. The employed modifications changed the

biological activities of these quinolone drugs, from antibacterial to cytotoxic agents. The most expressive result was obtained for the *O*-methyl oxime derivative (a 6-fluoro-4-oxo-quinoline derivative, compound **4**, Figure 13) that showed 95- fold increased activity (IC_{50}=2.5 μM) in MCF-7 cells (breast cancer), compared to its parent quinolone, norfloxacin (IC_{50}=238 μM).[69]

Another group compared the cytotoxic activity of norfloxacin with that of other quinolone derivatives, concluding that compound **5** (Figure 13), bearing a heterocyclic benzoxazole at 3-position, a 3-methylpiperazin-1-yl side chain at 7- position and a fluorine atom at 6 and 8-position, was the most potent derivative against the three cancer cells lines tested (oral epidermal, ovarian and hepatocellular carcinoma).[70]

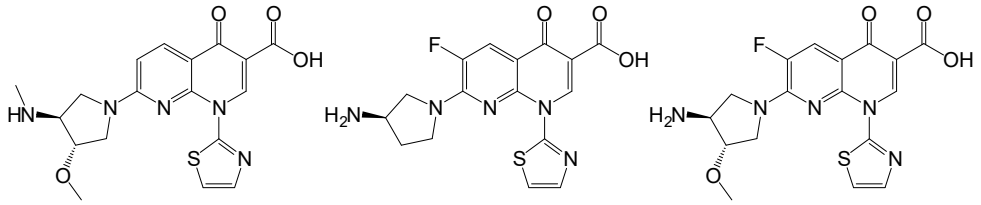


4 **5**

Figure 13. Structural representation of the *O*-methyl oxime derivative **4**, studied by Foroumadi *et al.,* and of 4-oxo-quinoline derivative **5**, studied by You *et al.*.

Over the years, studies showed that different quinolone analogues acted by distinct mechanisms on topoisomerase II: formation of a cleavable complex, which can be detected as DNA double strand breaks with formation of small toxic DNA fragments [7,71,72] and, on another step of the topo II action mechanism, prior to the formation of the cleavable complex, a possible intercalation with DNA. For the last mechanism of action a different pharmacophore could be considered, which is characterized mainly by an aryl substituent at N-1 and cyclic substituents at C-7.[72] **Vosaroxin** (Figure 14) is one of the analogues with these characteristics and is currently under clinical trials for use against acute myeloid leukemia.[73]

Firstly, compounds **6** and **7** (Figure 14) demonstrated good activity against several human tumor cell lines. Later, the same authors have proposed modifications at C-5, C-6 and C-7 positions and determined that the presence of the fluorine atom at C-6 is dispensable for the antitumor activity, leading to the drug **Vosaroxin**. For example, the 6-unsubstituted analogue of compound **6** was two times more potent than the 6-fluoro-1,8-naphthyridine (**6**), marking a significant difference in relation to quinolone antibacterials where the fluorine atom at this position has been shown to be instrumental for enhanced activity.[74]



Vosaroxin **6** **7**

Figure 14. Structural representation of **Vosaroxin** and its derivatives **6** and **7**, studied by Yasunori Tsuzuki *et al.*.

2.5. Quinolones with antiviral activity

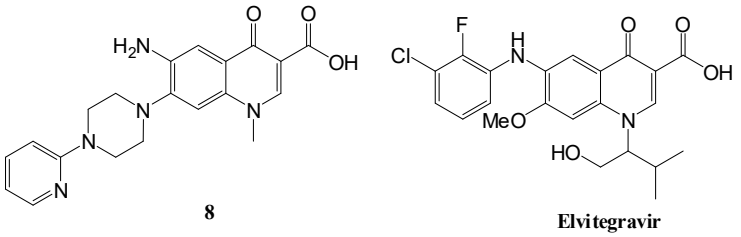
2.5.1. Quinolones as drugs against HIV

Viral infections are still a major health issue worldwide. The HIV (Human Immunodeficiency Virus) represents one of the most dangerous viruses affecting public health, since it causes an incurable disease and more than 36 million people are currently infected.[75] Although therapeutic advances transformed this deadly disease into a chronic one, many issues regarding HIV therapy remain, including drug resistance, that promotes gradual loss of efficacy of highly active antiretroviral therapy combinations, often due to poor

patient compliance, and cost.[76] Integrase inhibitors emerged recently as a novel anti-HIV drug class, and quinolone-type compounds play an important role in this class since they showed good activity against the viral DNA strand transfer step mediated by integrase enzymes (IN).[77]

A series of 6-aminoquinolone compounds were evaluated by Chow *et al.* [46] for their *in vitro* activity against HIV-1. Compound **8** (EC_{50}=0.1 μM, Figure 15) was the most active in inhibiting HIV-1 replication on *de novo* infected C8166 human lymphoblastoid cell lines.[78]

More recently, quinolone antibiotic derivatives proved to be potent against the strand transfer step of integrase activity and the use of the drug **Elvitegravir** (Figure 15) was approved for this indication.[79,80] Moreover, the quinolone scaffold also showed interaction with viral Reverse Transcriptase (RT), which makes it a promising structure for dual-mechanism development[81] or solely against viral retro-transcriptase.[82]



8 **Elvitegravir**

Figure 15. Structural representation of the 6-aminoquinolone **8** with potential interest against HIV-1, studied by Shibagaki *et al.*, and of **Elvitegravir**.

Additionally, studies show another promising use for the quinolone class in anti-HIV therapy, with the discovery of a strong interaction of 6-desfluoroquinolones with the TAR region of viral RNA, inhibiting its interaction with Tat protein, which is essential for the viral RNA transcription process.[83,84]

2.5.2. Quinolones as drugs against HSV and HCV

Several viral infections for which therapeutic response is not sufficient to provide successful cures continue to affect populations worldwide. Hepatitis C (HCV) and *Herpes simplex* (HSV) viruses represent two examples where novel and more efficient therapeutic tools are needed for an improved outcome. Current treatment for HCV infections consists of a combination of once-a-week subcutaneous pegylated Interferon (peg-IFN) alpha injections and twice-daily oral Ribavirin. However, these fail to provide full treatment in more than 50% of patients and the incidence of severe adverse effects is high.[85]

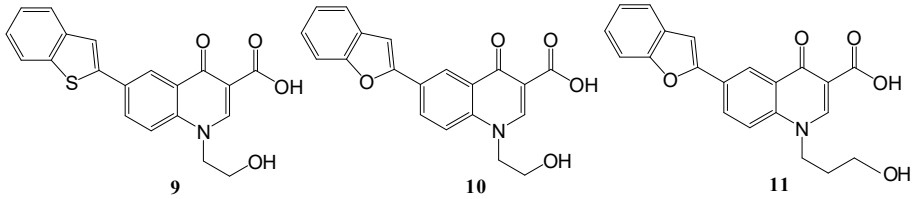
HCV NS5B polymerase enzyme displays a high active-site similarity with HIV integrase's active site.[86] Since 4-quinolone-3-carboxylic acids exhibit such good activities against HIV integrase, they could also represent a promising scaffold for interaction with HCV polymerase.

However, in this case structure-activity relationships show that best anti-HCV activity is achieved through substitution at C-6 with aromatic moieties, while substitution at C-7 position seems to be detrimental for activity. Given the positive results obtained from these molecules, it is of main interest to further explore the 4-quinolone-3-carboxylic acid scaffold for use in this field.[87]

In studies carried out by Chen *et al.*, it was observed that quinolone derivatives with a C-7 substitution pattern were inactive, whereas some isomers substituted at C-6 showed significant activity, especially those bearing an aromatic group as substituent. Relatively to the N-1 position, unsubstituted or simple alkyl N-1 substituted analogues showed no activity while hydroxyethyl and hydroxypropyl groups were substantially better for inhibition. The best inhibitory activity was achieved with 2-benzothiophene **9** and 2-benzofuran **10**, **11** substituted analogues (Figure 16**)**. Notably, the N-1 hydroxypropyl substituted analogue **11** was considerably less potent. Nevertheless, the best compounds **9** and **10** clearly showed better inhibitory activity toward HCV than Ribavirin, suggesting that the quinolone-3-carboxylic acid scaffold bears potential for antiviral discovery against HCV.[87]

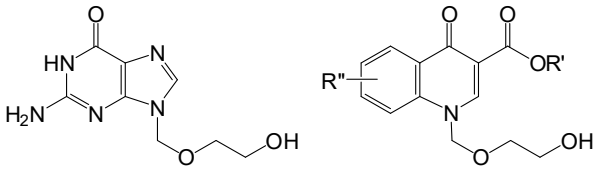
HSV-1 virus is causative of facial, oral and sometimes genital lesions in humans and its current treatment relies on the use of oral or topic antiviral drugs, such as **Acyclovir** (Figure 17), Famciclovir, and

Valacyclovir. Drug-resistance development by the HSV virus raises the need for new alternative treatments, effective against resistant strains.[88] Quinolone-based acyclovir analogues are a promising class of novel anti-HSV-1 drugs. These maintain the 1-(2-hydroxy-ethoxy) methyl substituent, but alter the purine core to a quinolone one, allowing for small substituents at C-6 or C-7.[89] An evaluation of the antiviral activity of quinolone carboxylic acids **13** and their corresponding esters **12** showed that almost all of the compounds reduced the viral load in 70-99%, being the acids, in general, more effective inhibitors than their corresponding esters.[89]



9 **10** **11**

Figure 16. Structural representation of the quinolone derivatives active against HCV strains.

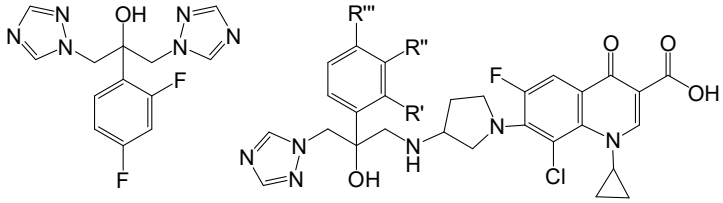


Acyclovir **12 (R': Et), 13 (R': H)**

Figure 17. Structural representation of **Acyclovir** and of its quinolone-based analogues.

2.6. Quinolones with antifungal activity

Quinolones were also shown to act against fungal infections, either by their synergistic effect with other antifungal drugs[14,90] or as hybrid drugs, as is the case of compound **14**, a scaffold combining **Ciprofloxacin** as the 4-oxo-quinoline moiety and a derivative of the known antifungal **Fluconazole** (Figure 18) linked to the 7-position of the quinolone core. This position is known to allow for chemical diversity and the possibility of optimization associated with broad spectrum and good pharmacokinetic properties, as reported by several authors.[14,91-93]



Fluconazole **14**

Figure 18. Structural representation of **Fluconazole**, and of hybrid drugs developed by its fusion with **Ciprofloxacin 14**.

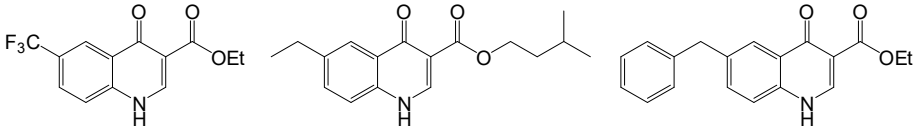
The bioactive assays on compounds **14**, performed by Yan Wang *et al.*, indicated that most of the target compounds displayed broad antimicrobial spectrum and good antifungal (and antibacterial) activities, with MIC values ranging from 0.25 to 2 μg/mL against all the tested strains, this translating in similar or even better efficiency, when compared with reference drugs such as **Fluconazole**.[91]

2.7. Quinolones with neurologic activity

2.7.1. Quinolones as drugs for the treatment of anxiety

Γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain. This neurotransmitter binds to its receptors, ionotropic $GABA_A$ and $GABA_C$, and metabotropic $GABA_B$, thus promoting its inhibitory effect. $GABA_A$, an ion-channel coupled receptor, has a pentameric structure with variable α, β and γ subunits, and is therefore responsible for several activities in the brain, such as sedation, anxiolytic activity and anterograde amnesia, depending on the subunit subtype.[94]

Currently available drugs used for stimulation of the $GABA_A$ receptor, to provide sedative, anticonvulsant, muscle relaxant, and anxiolytic effects, are mostly benzodiazepines, which are non-selective and hence present many adverse effects. Kahnberg *et al.* were the first group to investigate a quinolone for its GABAergic activity, during the study and validation of the pharmacophore model proposed for the benzodiazepine binding site,[95] and further improved the structure to obtain a compound with best interaction. Quinolones were shown to interact with $GABA_A$ receptors in an analogous way to benzodiazepines, by interacting with the same binding pocket.[96] From the wide diversity of studied compounds, compound **15** (Figure 19), a 4-oxo-quinoline derivative, stood out as the most active compound. The main pharmacophoric features include the carbonyl moieties at C-3 and C-4, which act as hydrogen bond acceptors, and the hydrogen bond donor amine at position 1.[95] Further modifications to the core structure led to the development of several active analogues, including two α1 selective compounds **16** and **17 (**Figure 19), which is the subunit responsible for sedation.[96]



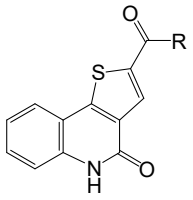
15 **16** **17**

Figure 19. Structural representation of quinolone derivatives that showed potential for the treatment and control of anxiety.

2.7.2. Quinolones as drugs for the treatment of Alzheimer's disease

Alzheimer's disease (AD) is one of the most dreaded chronic neurological disorders and leading cause of incapacity and death in elderly populations. Current treatments provide mainly symptomatic relief rather than disease modification, which calls for urgent development of disease-modifying therapies. Since AD is a multifarious disease, several therapeutic approaches are being studied for the development of an effective cure. Amyloid beta and tau proteins represent the two classical hallmarks in Alzheimer's disease and are the main targets under evaluation for their therapeutic potential.[97]

Chatterjee *et al.* reported the first quinolone active against tau hyperphosphorylation in Alzheimer's disease, acting by inhibition of the cdk5-p25 complex, where the quinolinic core scaffold bearing a carbonyl at C-2 appeared to be essential. Substitutions at C-3 and C-4 with a thieno ring structure and further side chain elongation led to several active and selective compounds, making scaffold **18** (Figure 20) a promising lead for further investigations in this field.[98]



18

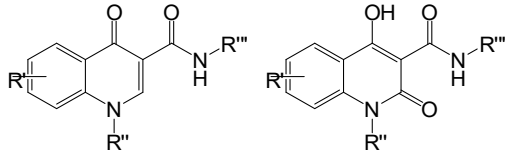
Figure 20. Structural representation of a quinolone-based pharmacophore active against the cdk5-p25 complex in Alzheimer's disease.

2.7.3. Quinolones with antinociceptive activity

Cannabinoid receptors participate in several physiological and pathological processes, including neuronal development, memory, anxiety and neurological disorders, ischemia, nociception, immunomodulation and inflammation, hormone release and action, bone formation, energy metabolism, cardiovascular, respiratory and reproductive functions, as well as cellular functions, such as cell architecture, proliferation, motility, adhesion and apoptosis. These receptors show potential in drug development, since their modulation can result in treatment or improvement of a large number of diseases and disorders.[99-102]

Pain is a complex phenomenon, frequently occurring in response to different neurologic stimuli, associated with actual or potential tissue damage.[103] Current treatment options for pain are limited and often exhibiting low efficacy and high range of adverse effects, as well as elevated costs.[104] Stimulation of cannabinoid receptors led to significant reduction of an array of different types of pain, including acute and chronic inflammatory pain, post-operative pain, cancer pain and neuropathic pain.[105]

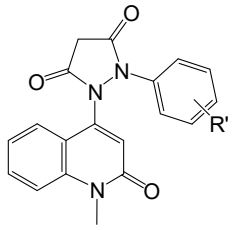
Pasquini *et al.* proposed the synthesis of quinolone derivatives and the study of their anti-nociceptive activity and possible anti-inflammatory effect as selective and potent agonists of the type 2 cannabinoid receptors (since CB1R, localized in the CNS, appeared to be associated to adverse effects upon stimulation). Initially the group started to explore the 4-quinolone-3-carboxamide scaffold **19** (Figure 21),[106,107] but later discovered improved activity and selectivity from 4-hydroxy-2-quinolone derivatives **20** (Figure 21) substituted at positions 6 and 7 and with variations on the carboxamide side chains. They showed that alkyl side chains at C-6 and fluoro substitution at C-7 resulted in best affinity, although C-6 substitution proved to be more favorable than the C-7 fluorination. However, N-1 substitutions, although allowing modulation of pharmacokinetic properties, proved to result in poorer activities and selectivity.[108]



19 **20**

Figure 21. Structural representation of 4-oxo-quinoline **19** and 4-hydroxy-2-oxo-quinoline **20** pharmacophores, developed by Pasquini and Mugnaini's research group.

Conversely, given the versatility of the 2-quinolones and pyrazolidine 3,5 diones, Tiwari *et al.* decided to fuse these structures, aiming for a high antinociceptive activity in Albino-Swiss mice. This study provided evidence that selected conjugates linking a 2-quinolone to a 3,5-pyrazolidinedione **21** (Figure 22) affect pain, the effect being more pronounced for derivatives bearing electron donating substituents (the pocket at the binding site may possess electron withdrawing groups). Although the mode of action was not established, comparable results to those available for preexisting analgesic and anti-inflammatory agents, such as NSAIDs, indicate that these compounds may also inhibit the release of endogenous pro-inflammatory mediators.[109]

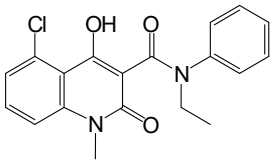


21

Figure 22. Structural representation of the 2-oxo-quinoline fused 3,5-pyrazolidinedione scaffold.

2.8. Quinolones with immunomodulatory and anti-inflammatory activities

Fluoroquinolones have for long been associated to an immunomodulatory effect in humans treated for bacterial infections. These compounds were shown to alter bacterial adherence and colonization properties to epithelial surfaces, induce and improve phagocytic processes against bacterial components and intraleukocytic killing,[110] stimulating further exploration of this chemotype for its immunomodulatory effect. Several compounds were since designed and studied for applications in this field. **Laquinimod 22** (Figure 23) is a 4-hydroxy-2-oxo-quinoline-3-carboxamide currently used in the treatment of multiple-sclerosis due to its immunomodulatory and anti-inflammatory properties.[111] This compound acts on several regulatory levels, including suppression of inflammatory response *via* Transforming Growth Factor beta and Nuclear factor κ B pathways, as well as of cell movement processes, such as adhesion, migration and leukocyte extravasation.[112]

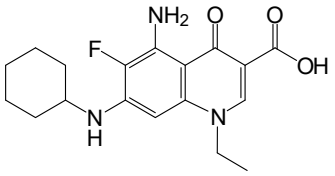


22

Figure 23. Structural representation of the immunomodulating drug **Laquinimod 22**.

2.9. Quinolones with metabolic activity

Metabolic disorders like diabetes or obesity are multifactorial diseases involving, apart from the aforementioned cannabinoid receptors, dysregulation of several other physiological pathways. Forkhead box protein O1, FoxO1, is an essential mediator of hepatic gluconeogenesis and insulin signaling, and overall glucose and insulin level regulation, resulting in general impairment of glucose tolerance and insulin sensitivity, which are main features present in metabolic diseases. **AS1842856** (Figure 24) is a 5-amino-7-(cyclohexylamino)-1-ethyl-6-fluoro-4-oxo-quinoline-3-carboxylic acid that acts by selectively inhibiting FoxO1, with further inhibition in glucose production by the liver and adipogenesis.[113,114] Its precise action mechanism and binding properties have not yet been clarified, but the good activity results demand a more profound study of this lead to allow for better therapeutic effects in patients with metabolic diseases.



AS1842856

Figure 24. Structural representation of **AS1842856**.

The protein tyrosine phosphatase 1B (PTP1B) is another important mediator of insulin regulation, acting by dephosphorylation and subsequent inactivation of several essential proteins, including insulin receptors and insulin receptor substrates. This protein was shown to be non-essential for survival and normal development in mice, indicating that its inhibition isn't physiologically deleterious, which renders it a promising structure for the treatment of metabolic diseases that result from insulin dysregulation.[115]

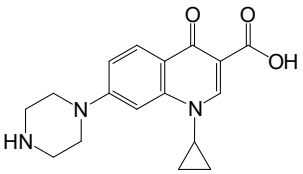
Zhi *et al.* showed that selected 4-quinolones possess important features for interaction with the PTP1B active site, including the hydrophobic aryl group in the quinoline core and the di-keto moiety, also present in the 4-quinolone-3-carboxylic acid scaffold. Additional modifications to the core enhanced activity, namely the introduction of bulky lipophilic aromatic groups at C-6 and small alkyl groups at N-1, or double aromatic substitutions at N-1/C-6.[116]

Subsequent discovery of an additional binding pocket adjacent to the catalytic active site led the group to link a salicylic acid moiety to the C-6 position of the quinolone derivatives, obtaining bifunctional agents which interact with both pockets, promoting improved activity and selectivity.[116]

2.10. Quinolones as drugs for the treatment of ischemia

As mentioned above, cannabinoid receptors mediate a variety of physiological processes in which ischemia is also included. These receptors, particularly CB2R, have the ability to protect from and reduce ischemic injury in several affected organs, acting through unknown mechanisms, as disclosed by different authors.[117,118] Murikinati *et al.* have shown that selective CB2R agonists play an important role in reduction of ischemia through a mechanism involving neutrophil migration, adherence and chemotaxis.[102] Given that quinolones show very good activities and interactions with CB2 receptors, it is likely that this drug class will provide interesting leads to further explore in this field.

Also, quinolone antibacterials have shown to augment cell survival ratios in hypoxic environments, which led Parks *et al.* to study the activity of quinolone derivatives in anti-ischemic processes. The authors developed several ciprofloxacin analogues, leading to the structure **SQ-4004** (Figure 25) that showed best anti-ischemic activity and selectivity in hypoxic environments towards eukaryotic cells rather than in bacterial cells.[119]



SQ-4004

Figure 25. Structural representation of the most active anti-ischemic ciprofloxacin derivative **SQ-4004**.

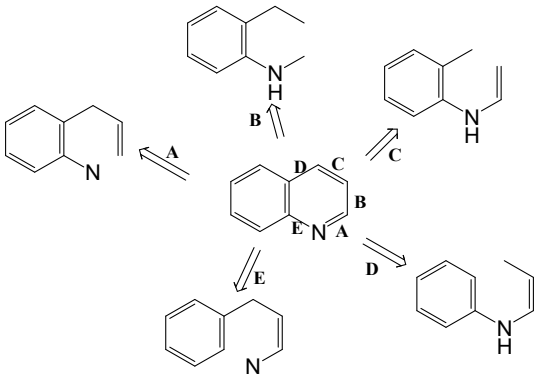
3. Synthesis of quinolones

Given the range of applications of quinolones in medicinal chemistry, highlighted in Section 2, the availability of mild, high yielding, selective and versatile synthetic routes to this class is a matter of upmost relevance. However, we think that this area requires more investment.

Skraup reported on the first formal synthesis of the quinoline core (part of the structure of quinine), over a century ago.[120] Later, several variations to the original Skraup synthesis and new methods have been reported to afford quinoline derivatives, such as the Combes reaction,[121] the Friedlander synthesis,[122] the Doebner-von Miller synthesis,[123] the Pfitzinger-Borsche reaction[124] and the Povarov reaction.[125] These methods enabled the preparation and optimisation of chemically diverse libraries of quinoline derivatives, through alterations in the patterns of substitution on the quinoline core and in the chemical nature of substituents.

Synthetic approaches for 2-oxo- and 4-oxo-quinoline synthesis, from a variety of starting points, have been developed and optimized. These can be classified according to the bond or bonds that complete the ring-closure and the method used to increase the carbon chain length. A retrosynthetic diagram showing the possibilities for construction of the quinolone bicyclic system is represented in Scheme 1 (for the quinoline core) and some of the available methodologies are described in following subtopics.

Scheme 1 presents the five fundamental strategies available for the synthesis of the quinoline core (Scheme 1), all others being variations of these. In **Type *A*** the linkage is effected between the nitrogen atom and the α-carbon atom; in **Type *B*** the ring-closure occurs between the α- and β-carbon atoms; in **Type *C*** the bonding is effected between the β- and γ-carbon atoms; in **Type *D*** the ring closure is effected through a linkage between the γ-carbon atom and the benzene nucleus; and in **Type *E*** the ring closure is achieved by bond formation between the benzene nucleus and the nitrogen atom. There are approaches which do not fit into this simple classification, as is the case of the introduction of carbon atoms α and β (combination of Types *A* and *C*). The different types and combinations of strategies leading to the synthesis of 2-oxo- and 4-oxo-quinolines will be discussed below.

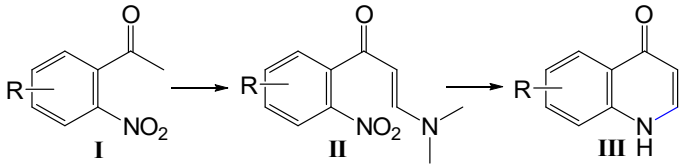


Scheme 1. Retrosynthetic approaches to the preparation of the quinoline scaffold, following the formation of each bond (**Types *A*** to ***E***).

3.1. Type *A*

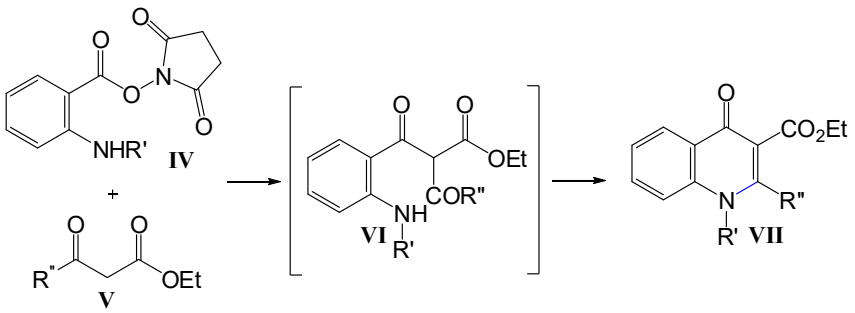
For ring closure to 4-oxo-quinolines through formation of bond *A*, two basic methods were proposed, by Koskinen[126] and Igglessi-Markopoulou[127] groups.

Tois *et al.* described the synthesis of 2,3-unsubstituted 4-oxo-quinolines **III** from an enamine **II** (Scheme 2), under reducing conditions, in cyclohexene, using 10% Pd on charcoal as catalyst. Enamine **II** may be synthesized by condensation of *o*-nitroacetophenone **I** with *N,N*-dimethylformamide dimethylacetal $(CH_3)_2NCH(OCH_3)_2$, in *N,N*-dimethylformamide (DMF).[126]



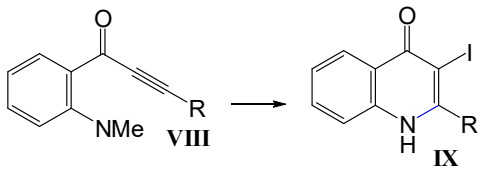
Scheme 2. Schematic representation of the synthetic approach to 2,3-unsubstituted 4-oxo-quinolines, proposed by Koshinen.[126]

Igglessi-Markopoulou and Mitsos *et al.* proposed a methodology for the synthesis of 3-ethoxycarbonyl 4-oxo-quinolines **VII** (Scheme 3) involving the acylation of β-keto esters **V** with *N*-hydroxysuccinimide esters of anthranilic acids **IV** to form conjugates **VI**, which progress spontaneously to the corresponding quinolone 3-esters, through cyclisation.[127]



Scheme 3. Schematic representation of the synthetic approach to 3-ethoxycarbonyl 4-oxo-quinolines, proposed by Igglessi-Markopoulou and Mitsos group.[127]

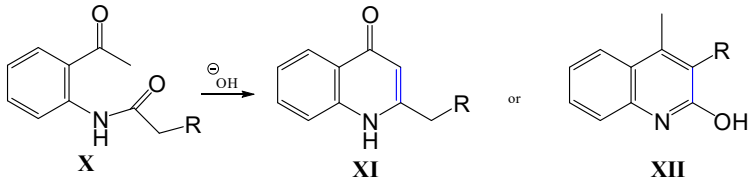
The ICl-induced cyclisation of heteroatom-substituted alkynones **VIII** provides a simple approach to synthesize 3-iodo-4-oxo-quinolines and analogues (Scheme 4), in good to excellent yields. This process, proposed by Chengxiang *et al.*, involves mild conditions, tolerating various functional groups at the structure. 3-iodo-4-oxo-quinoline derivatives **IX** can be subsequently used as substrates in palladium-catalyzed transformations, enabling the increase in complexity and chemical diversity.[128]



Scheme 4. Schematic representation of the synthetic approach to 3-iodo 4-oxo-quinolines, proposed by Chenxiang *et al*.[128]

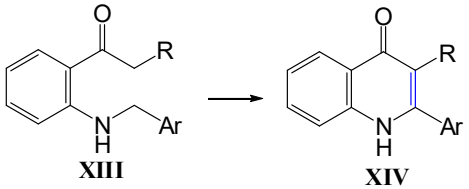
3.2. Type *B*

The Camps cyclisation (Scheme 5) is the main method for ring closure to afford quinolones through formation of bond *B*. This base catalysed ring closure methodology provides an access to 2-substituted 4-oxo-quinolines **XI**. However, depending of the conditions and starting material, the same methodology can also be used to prepare 2-hydroxy-quinolines **XII**, which could potentially be converted into the corresponding 2-oxo-quinoline tautomers.[129]



Scheme 5. Schematic representation of the Camps cyclisation methodology.[129]

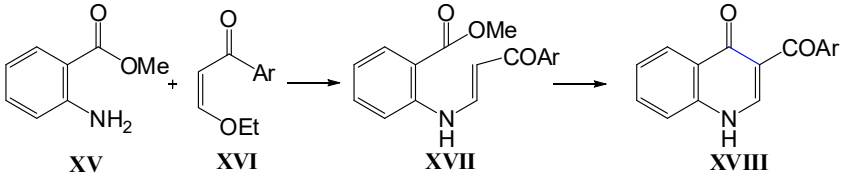
Another method involving ring closure through the formation of bond *B* (Scheme 6) has been used for the synthesis of 2,3-disubstituted 4-oxo-quinolines, more specifically of 2-aryl-quinolin-4(1*H*)-ones **XIV**. This methodology, based on oxidative intramolecular Mannich reaction using of TEMPO as oxidant and KOtBu as the base, is considered as a simple and direct access to quinolones from readily available *N*-arylmethyl-2-aminophenylketones **XIII**.[130]



Scheme 6. Schematic representation of the synthetic approach to 2-aryl-quinolin-4(1*H*)-ones.[130]

3.3. Type *C*

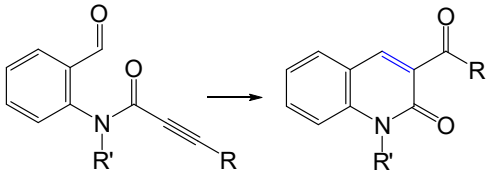
A Michael-type addition (Scheme 7) of methyl anthranylate **XV** to β-ketonic enol ethers **XVI** may provide access to 3-aroyl-4-oxo-quinoline derivatives **XVIII** through formation of bond *C*. The reaction is thought to involve a Michael adduct **XVII** that, through ring closure facilitated by heating at reflux in diphenyl ether and in the presence of sodium methoxide, originates the 4-oxo-quinolines **XVIII**.[131]



XV **XVI** **XVII** **XVIII**

Scheme 7. Schematic representation of the synthetic approach to 3-aroyl 4-oxo-quinolines, proposed by Hénichart *et al.*[131]

A $Pd(OAc)_2$-catalyzed cyclisation strategy through formation of bond *C* can be used for the preparation of 2-oxo-quinolines (Scheme 8). This methodology allows the conversion of alkynes (more specifically *N*-(2-formyl-aryl)alkynamides **XIX**) to the corresponding 2-oxo-quinoline derivatives **XX**, *via* oxypalladation.[132]

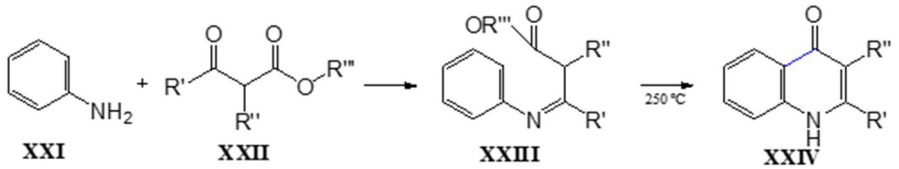


XIX **XX**

Scheme 8. Schematic representation of the synthetic approach to 2-oxo-quinolines from *N*-(2-formyl-aryl)alkynamides.[132]

3.4. Type *D*

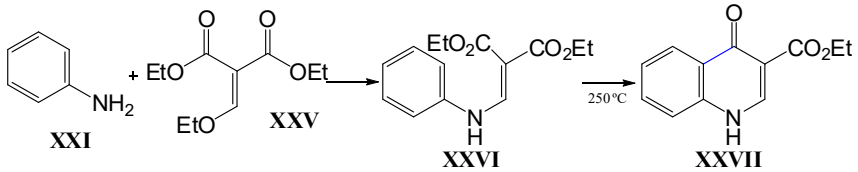
The Conrad-Limpach methodology (Scheme 9) is one of the main strategies for the synthesis of 4-oxo-quinolines through the formation of bond *D*, whereby 4-oxo-quinolines **XXIV** result from the condensation of anilines **XXI** with β-keto esters **XXII**. The reaction affords an imine **XXIII** as intermediate, which must be heated at around 250 ºC for the ring closure by formation of the bond *D*. This methodology can produce quinolones with various substitution patterns, defined by the nature and pattern of substitution of the starting compounds, aniline and the β-keto ester.[133]



XXI **XXII** **XXIII** **XXIV**

Scheme 9. Schematic representation of the Conrad-Limpach strategy for 4-oxo-quinoline synthesis.[133]

The reactions based on ring closure through bond *D* for the preparation of 4-oxo-quinolines comprise several methods. The Gould-Jacobs methodology (Scheme 10), published in 1939, is an adaptation of the Conrad-Limpach synthesis, involving the thermally driven intramolecular cyclisation of an enamine **XXVI** to prepare the final 4-oxo-quinoline 3-esters **XXVII**. The reaction is normally conducted at 240-250 ºC.[134]

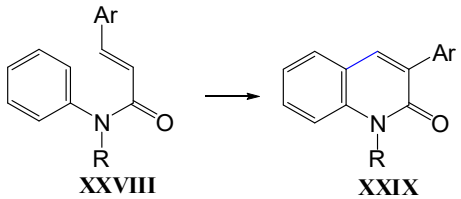


XXI **XXV** **XXVI** **XXVII**

Scheme 10. Schematic representation of the Gould-Jacobs methodology for quinolone synthesis.[134]

For the synthesis of 2-oxo-quinolines based on ring closure through formation of bond *D*, several methods could be employed and, from them, two examples will be presented.

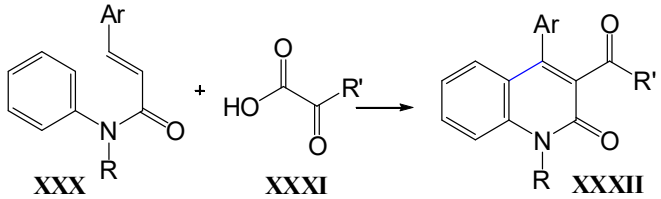
In 2013, Liu *et al.* described a general and efficient reaction that combines a metal-free oxidative $C(sp^2)$-$C(sp^2)$ bond formation and an exclusive 1,2-aryl migration (Scheme 11). Following this methodology, reaction of a readily available *N*-methyl-*N*-phenylcinnamamide **XXVIII** with phenyliodine bis(trifluoroacetate) (PIFA) in the presence of a Lewis acid (for example BF_3) provides 3-aryl-2-oxo-quinoline derivatives **XXIX** in good yields.[135]



XXVIII **XXIX**

Scheme 11. Schematic representation for the strategy proposed by Liu *et al*. for the synthesis of 2-oxo-quinolines through the formation of bond *D* and 1,2-aryl migration.[135]

A silver-catalyzed methodology has been developed for the one step synthesis of 3,4-disubstituted 2-oxo-quinolines **XXXII** bearing a carbonyl moiety at position 3 and an aryl substituent at position 4 (Scheme 12). This strategy provides a practical, highly efficient and straightforward route to 3,4-disubstituted 2-oxo-quinolines, through an intramolecular radical cyclisation in aqueous solution, with formation of bond *D* and acylation at C-3.[136]



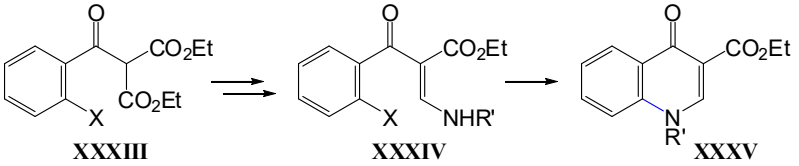
XXX **XXXI** **XXXII**

Scheme 12. Schematic representation of the strategy proposed by Mai *et al*. for the synthesis of 3,4-disubstituted 2-oxo-quinoline derivatives.[136]

3.5. Type *E*

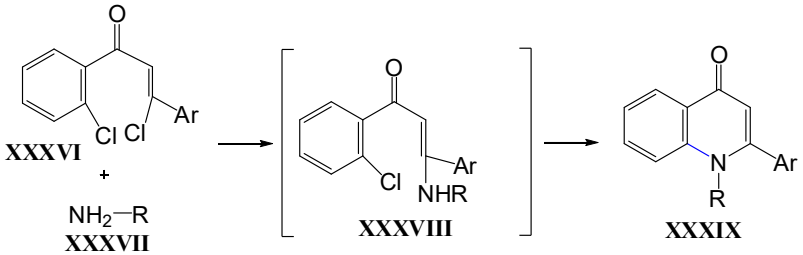
Variously substituted 4-oxo-quinolines can be obtained by the formation of bond *E* and some examples have been reviewed. Scheme 13 presents an example of application of a methodology that includes multistage reactions with successive transformations, in which amino vinyl phenyl ketones **XXXV** are formed (for example by hydrolysis, decarboxylation and transamination reactions from **XXXIII**). Ring closure, at the last stage, involves the halogen atom (X) at the *ortho* position of the aryl substituent in **XXXIV**. The cyclisation step to the formation of 4-oxo-quinolines **XXXV** can be achieved in the presence of various reagents and conditions, for example *via* copper-catalysed hetero-cyclisation. This strategy allows the introduction of a variety of useful functionalities at C-3, including ester, keto, cyano, and chlorine substituents.[137,138]

A multistage strategy (Scheme 14) can also be employed for the synthesis of 4-oxo-quinoline derivatives, enabling chemical diversity at C-2 position (with an aryl moiety). This example involves the intermolecular Michael addition of an amine **XXXVII** to a (*Z*)-β-chlorovinyl ketone **XXXVI** followed by elimination of a chloride anion, providing enamine intermediates **XXXVIII** that can be transformed into quinolin-4(1*H*)-one **XXXIX** products by a palladium-catalyzed intramolecular *N*-arylation, in a tandem one-pot manner, with good to excellent yields.[139] Different scientific groups have studied this kind of strategy and adaptations of this multistage example have been reported, allowing the synthesis of chemically diverse 4-oxo-quinolines.[140,141]



XXXIII **XXXIV** **XXXV**

Scheme 13. Schematic representation of the synthetic approach to quinolones involving multistage reactions and cyclisation by formation of bond *E*.[137,138]



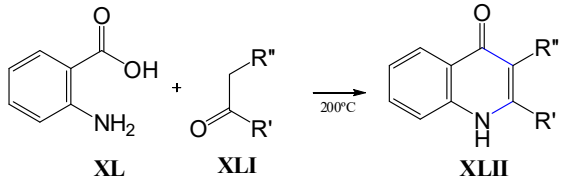
XXXVI + **XXXVII** **XXXVIII** **XXXIX**

Scheme 14. Schematic representation of a multistage synthetic strategy to 2-aryl-4-oxo-quinolines.[139]

3.6. Combination of *multi-bond* formation

As mentioned before, some synthetic approaches to quinolones do not fit into the simple classification of a unique bond formation required for ring closure.

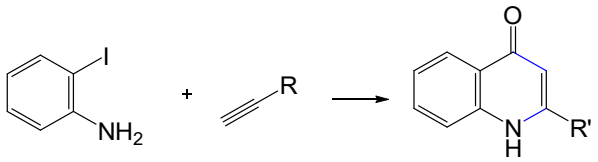
In 1894, Niementowski *et al.* described the methodology for 4-oxo-quinoline synthesis (Scheme 15), consisting in the thermal condensation (at 200 ºC) of anthranilic acids **XL** with ketones **XLI**, obtaining 2,3-substituted 4-oxo-quinolines **XLII** through the formation of bonds *A* and *C*.[142]



XL **XLI** **XLII**

Scheme 15. Schematic representation of the Niementowski methodology for 4-oxo-quinoline synthesis.[142]

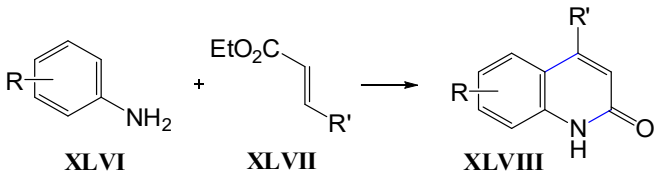
In 2015, Akerbladh *et al.* described the reactions between 2-iodoanilines **XLIII** and alkynes **XLIV** (Scheme 16), showing that the use of two different protocols leads to the synthesis of 2-substituted-4-oxo-quinolines **XLV**. Both methodologies were described as palladium-catalysed but, while in one method the reaction was carried out under microwave and heating system (120 ºC, 20 minutes), the other employs a gas-free one-pot two-step sequence (running at room temperature), allowing the use of sensitive substituents (for example nitro and bromide groups).[143]



XLIII **XLIV** **XLV**

Scheme 16. Schematic representation of the strategy to 4-oxo-quinolines by Akerbladh *et al.*.[143]

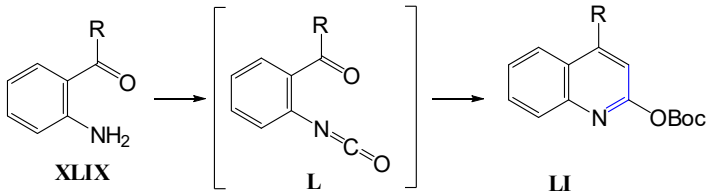
Also starting from simple anilines **XLVI** and based on a palladium-catalysed reaction, J. Wu *et al.* have described a cascade process strategy of C-H bond activation, C-C bond formation and cyclisation through the formation of bonds *A* and *D* (Scheme 17), enabling the synthesis of 2-oxo-quinolines **XLVIII**.[144] A metal-catalysed cyclisation (Ru-catalysed) has also been proposed in 2014 by Jeganmohan *et al.* for the synthesis of 2-oxo-quinolines, using anilides and propiolates or acrylates as starting materials.[145]



XLVI **XLVII** **XLVIII**

Scheme 17. Schematic representation of the strategy proposed by J. Wu *et al.* for the synthesis of 2-oxo-quinolines from anilines.[144]

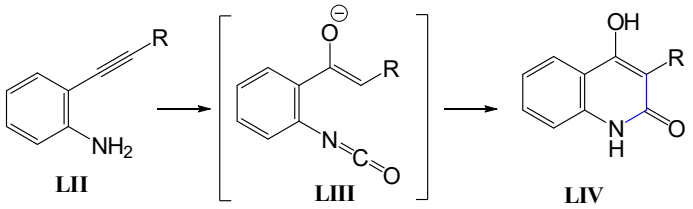
Recently, a novel strategy for the synthesis of 2-oxo-quinolines from 2-alkenylanilines **XIIX** was proposed by Huang *et al.* (Scheme 18). This methodology involves a DMAP-catalysed cyclisation with di-tert-butyl dicarbonate (Boc_2O), leading to formation of a tert-butyl quinolin-2-yl carbonate **LI**. This product is a versatile building block that can be easily converted into the corresponding 2-oxo-quinoline derivative.[146]



XLIX **L** **LI**

Scheme 18. Schematic representation of the strategy by Huang *et al.* for the synthesis of 2-oxo-quinolines from 2-alkenylanilines.[146]

Among quinoline derivatives, 4-hydroxy-2-oxo-quinolines **LIV** have attracted special attention due to their biological properties. This class of compounds can be accessed under mild reaction conditions, in the presence of a silver salt as catalyst. As represented in Scheme 19, it is possible to obtain a range of chemically diverse 4-hydroxy-2-oxo-quinolines from from *o*-alkynylanilines **LII**.[147]



LII **LIII** **LIV**

Scheme 19. Schematic representation of the strategy proposed by Ishida *et al.* for the synthesis of 4-hydroxy-2-oxo-quinolines.[147]

4. Liabilities of quinolones

Previous sections highlight the pharmacological properties and versatility of quinolones. However, some liabilities have been reported for different groups, encompassing the synthesis, structure

and properties of quinolone derivatives. Clinically, the liabilities presented by quinolones are especially related with side effects, food-drug or drug-drug interactions and the development of resistance. On the chemistry side, deeper structural studies are required for a better understanding of their reactivity and biological activity. Also, efforts are needed towards the development of reliable synthetic routes to quinolones that enable the easy preparation of diverse libraries of compounds, from which newer and better leads can be selected. This section emphasizes the major liabilities encountered on the application of quinolones in medicinal chemistry.

4.1. Toxicity and side effects

Some toxicity and side effects have been attributed to quinolone-based drugs, especially those commercially available as antibiotics, calling for the intervention of regulatory agencies.

Although adverse reactions are uncommon, in 2016 the FDA restricted the use of fluoroquinolone antibiotics for specific infections, because of the potential for risks of peripheral neuropathy, central nervous system effects, and cardiac, dermatologic, and hypersensitivity reactions in adults.[148,149]

Studies for animal toxicity performed for the first quinolone derivatives revealed their propensity to cause inflammation and, subsequently, damage and destruction of joints in canine puppies.[150] Although no compelling published evidence to date supports the occurrence of sustained injury to developing bones or joints in humans, FDA suggests the possibility of increased musculoskeletal adverse events (tendinopathy, arthritis, arthralgia and gait abnormality) during the treatment, but with no sequels signals after cessation.[148] Each quinolone has a different potential to cause cartilage toxicity and it depends of the drug's concentration in the cartilage. Quinolones form chelate complexes, for example with magnesium, and interfere at the cartilage matrix integrity.[151,152]

Beyond the musculoskeletal adverse events (most frequently reported with arthralgia), systematic reviews have reported that the use of fluoroquinolones in paediatric patients may lead to abnormal liver and renal function, nausea, white blood cell count derangements, vomiting, disorders of glucose homeostasis and rash (increment of photosensitivity; advise to use sun-protection measures).[153,154] The use of fluoroquinolone antibiotics may also lead to side effects on the central nervous system (seizures, headaches, dizziness, sleep disorders, hallucinations) and peripheral neuropathy.[155] Additionally, some studies suggested that the treatment using fluoroquinolones increases the risk of infection by *Clostridium difficile*.[156]

Cardiotoxicity of fluoroquinolones is another side effect described (especially in adults), since these drugs can prolong the QT interval.[157] This adverse effect takes us to another liability often related with the use of quinolones, their lack of selectivity.

4.2. Selectivity

The evaluation of selectivity is especially relevant when related with the development of compounds to be used in medicinal chemistry, as they may interfere with different targets, leading to undesired toxicity. Thus, medicinal chemists generally seek for target selectivity. However, occasionally the same compound can be considered for the treatment of more than one disease. For example, some quinolones have been investigated as potential drugs to treat malaria and also tuberculosis. In addition, compounds that are able to address more than one target could be more effective. For instance, selected quinolones are known to disrupt the mitochondrial electron transport chain of *P. falciparum*.[37] Some authors proposed that the compounds act by targeting the Q_o site of the bc_1 complex of *P. falciparum*[6] while others have hypothesized a different mode of action from the intended Q_o inhibition, proposing the Q_i site of the bc_1 complex and *Pf*NDH2 as other possible targets.[3,6,158-161] Nowadays, some quinolone derivatives are known as potential inhibitors of *Pf*NDH2 and bc_1 complex of *P. falciparum* (by binding at Q_o site), and this multi-target inhibition confers a benefit over the single-target inhibition, delaying the process of selection for drug resistance.[3,4]

Some compounds structurally similar to quinolone derivatives (4(1*H*)-pyridone chemotype **GW844520** and **GSK932121**, Figure 26), which have been optimized for antiplasmodial activity, emerged as promising leads but had to be withdrawn from development due to unexpected toxicity, attributed to cardiotoxicity and possibly due to the inhibition of the mammalian bc_1 complex, by binding at the Q_i site.[32,37,162,163] In this case, it might be possible to circumvent toxicity-related events by conferring selectivity to the drug (or drug candidate), this requiring structure optimization and, thus, additional synthetic work.

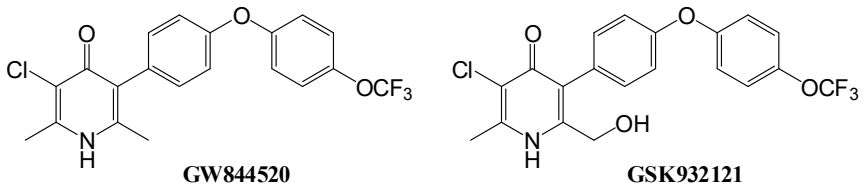


Figure 26. Structural representation of 4(1*H*)-pyridones **GW844520** and **GSK932121** docked for the Qi site of cytochrome bc1 in a previous study.

4.3. Resistance

In clinical practice, the development of resistance has been a concern since the approval of quinolones as antibacterial drugs. Multiple mechanisms of resistance have been described in some bacterial pathogens, including mutations leading to changes in the target enzymes DNA gyrase and DNA topoisomerase, as well as efflux pumps (transport proteins involved in the extrusion of toxic substrates) and alterations in membrane proteins.[164] It is expected that resistance phenomena will increase proportionally with the use of these antibiotics and there is a clear risk of resistance in patients exposed to repeated treatment courses.[165]

At the research and development level, the potential use of quinolones as antimalarials also faces the problem of the growing spread of resistance by *P. falciparum*, the most prevalent strain affecting mankind. Along the last four decades, *P. falciparum* has developed resistance to almost every available antimalarial drug, placing selection for resistance by the parasite as a major obstacle in the control of malaria.

The search for novel chemotherapeutics to circumvent the problem of resistance to antimalarials led to the approval of Malarone®, a combination of proguanil and atovaquone (a naphthoquinone), for the treatment and prevention of multidrug resistant malaria.[167] However, the first cases of Malarone® resistance were observed in Africa shortly after the introduction of Malarone®, and were linked to mutations at the cytochrome *b* structure.[33,167–169] Since some mutations associated to atovaquone resistance tend to confer cross resistance to other Q_o inhibitors (for example to 4-oxo-quinoline derivatives in SAR studies), the new and more cost-effective inhibitors of the bc_1 complex must overcome the resistance mechanisms.[32] Studies about this class have been reported by various authors, highlighting the works of Trumpower *et al.*,[169] O'Neill *et al.*,[162] Biagini *et al.*,[32] Beteck *et al.*[25] and Gomes *et al.*.[170]

In the 70's of last century, Ryley *et al.* noted problems related to fast resistance development and cross-resistance (with atovaquone) in rodent models, for some antimalarial quinolone 3-esters (ICI 56780 and ICI 60128, Figure 5).[30] However, recent research suggests that the cross-resistance for atovaquone resistant parasite strains of the quinoline derivatives could be significantly diminished by altering the nature of substituents at the quinolone core.[27,38] From the compounds prepared selected quinolones have been investigated and, although exhibiting excelent activity, did not circumvent totally the problem of atovaquone resistance, demonstrating some cross resistance with atovaquone. However, when tested in atovaquone resistant strains, only a 92 fold drop in potency was observed, representing a clear improvement over the 760-fold decrease in potency observed for atovaquone in the same atovaquone-resistant parasite strain.[6,161,171]

The fight against some of the most deadly infective diseases in the world remains a priority. Therefore, additional studies to circuvent the growing spread of resistance to quinolone-based drugs are mandatory, especially for those in use as antibacterial and antiplasmodial agents.

4.4. Drug-drug and drug-food interactions

When prescribing a drug for a specific disease it is important to consider potential interactions with other drugs and, in some cases, with food. The most common drug interaction mechanisms with clinically significant sequelae are due to inhibition of drug absorption, alterations in drug metabolism, distribution or excretion and addition of side-effects. Drug interactions that produce recognizable toxicity are more likely to be detected than those that result in reduced efficacy of the drugs. From the quinolone derivatives described in this chapter, fluoroquinolones are those with more studies reporting drug interactions, the more intense investigation in this sub-class is being ascribed to the extensive use of fluoroquinolones as antibiotics.

GI absorption of ciprofloxacin and several other quinolone antibiotics is reduced by the concomitant use of an antacid or mineral supplement (both containing aluminum, magnesium, calcium or iron). This interaction can be translated in therapeutic failure, for example due to chelation of the antibiotic with ingested metals, forming a complex that is poorly absorbed from the gastrointestinal tract and subsequently limits the access to the systemic circulation. For example, the bioavailability of ciprofloxacin has been reported to decrease by as much as 90% when administered with antacids containing aluminum or magnesium hydroxide.[172] Additionally, food products known to contain high concentrations of calcium (for example milk, yogurt or cereal or orange juice), should be avoided during the therapy with quinolone derivatives.[173,174]

Food, in general, may also reduce the oral absorption of fluoroquinolones, possibly by competing for the same influx pumps, reducing the bioavailability. Levofloxacin is the most affected by a concomitant administration with food, prolonging the time to peak concentration of the drug by 1 hour and decreasing the $C_{máx}$ by 14-25%.[175]

Drug interactions by modifications of hepatic metabolism can be related to either inhibition or induction of enzymes, especially from the cytochrome P450 (CYP 450) metabolic system, such as CYP3A4, CYP2D6, CYP2C, and CYP1A2. The inhibition of drug metabolism favors its bioavailability (drug accumulation over time) and may lead to an increment of toxicity. On the other side, the increase in drug metabolism may lead to treatment failure, if translated in a reduction of the level of the active drug.[173]

Several quinolone-derived drugs, such as ciprofloxacin, norfloxacin and nalidixic acid, may inhibit CYP1A2 and, therefore, the co-administration of these antibiotics with other drugs primarily metabolized by CYP1A2 results in increased plasma concentrations of these drugs and could lead to clinically significant adverse events of the co-administered drug. For example the concurrent administration of ciprofloxacin with theophylline (drug used in therapy for respiratory diseases, such as chronic obstructive pulmonary disease) may result in increased risk of a patient developing central nervous system (CNS) or other adverse reactions due to a significant increment of the theophylline's serum concentrations.[176] Quinolones may also increase the plasma concentrations and effects of caffeine, due to this inhibition, causing headache, tremor, restlessness, nervousness, insomnia, tachycardia, and blood pressure increases.[177,178]

The overlap or additive effect in terms of side effects is another kind of interaction with relevance for the use of quinolone derivatives in medicine. One example is the concomitant use of fluoroquinolones with other drugs known to prolong QT interval (for example, some anti-arrhythmics, tricyclic antidepressants, macrolides or antipsychotics), since fluoroquinolones may further prolong the QT interval, resulting in elevated risk of ventricular arrhythmias, including ventricular tachycardia and torsade de pointes.[179]

Although in the clinical practice more results about toxicity and interactions are obtained and reported, during the preliminary stages of the development of new drug candidates several studies might be performed to predict the toxicity profile and the potential to interact with other drugs or even food.

For example, in a recent project coordinated by Hong et al.[64] and aimed at the development of novel quinolones targeting the respiratory chain of *Mycobacterium tuberculosis*, some studies to determine the cytotoxicity towards HEPG2 were carried out, as a way to evaluate the hepatotoxicity of the potential drug candidates. None of the compounds tested were found to be cytotoxic and all exhibited good therapeutic indexes. From this study, MTC420 (Figure 12), demonstrated acceptable antituberculosis activity and favorable pharmacokinetic and toxicological profiles. In terms of *in vitro* CYP450 inhibition, this lead compound presented no inhibitory effect over CYP2C9, 2D6, 3A4 and 3A5, only showing some inhibitory capacity over CYP2C8. Therefore, the quinolone derivative MTC420 should not be problematic in relation to drug interactions arising from modifications of hepatic metabolism.[64]

4.5. Solubility, synthesis, characterization and pharmacokinetic profile

One of the major liabilities encountered during the design and development of novel quinolones with potential interest in medicine is related with problems of solubility. In general, quinolone derivatives are characterized by high melting points, possibly related with aggregation *via* π-stacking of aromatic ring systems, conferring planarity and poor solubility.[180]

In fact, the poor solubility of quinolones becomes prominent as early as the synthesis stage, often causing major problems associated to the purification and isolation of the products, for example by column

chromatography and/or recrystallization techniques. The poor solubility also renders the characterization of quinolones difficult, especially when a solubilisation phase is needed.[34-36] Different synthetic approaches to quinolone derivatives are often required to circumvent these problems, which may require additional steps. For example, chloroquinolines are often used as intermediate compounds, as they are more easily solubilized and isolated and may be subsequently converted into the corresponding quinolones.[34] However, some limitations were also ascribed to the preparation of chloroquinolines, which involves $POCl_3$ mediated cyclization: in some cases the reaction did not progress, or it was not possible to isolate a pure product, or unexpected products were obtained.[34]

For some quinolone derivatives in development the poor solubility may also interfere with the pharmacological activity, since it affects the pharmacokinetic profile of the potential drugs. To reach its target, the drug must pass through several membranes, beginning with the absorption in the stomach and small and large intestine (if orally administered). To pass across most membranes, the drug must be relatively non-polar, but, since it must be solubilized in biological media, it should also possess some polar characteristics. Usually, in medicinal chemistry, the logarithm of the partition coefficient (LogP) is used to assess the drug-likeness of a given molecule, as it is a well-established measure for the compound's hydrophilicity and/or hydrophobicity.[181]

Partition coefficients have a strong influence on pharmacokinetic properties of drugs and are useful to estimate their solubility, absorption and distribution of drugs within the body. However, LogP is not an accurate determinant of lipophilicity for ionisable compounds, because it only describes the partition coefficient of neutral molecules. This limitation can affect the research, since approximately 80% of the drugs are ionisable and are subject to the changing pH environments in the body.[181-183] For a drug to be orally absorbed, it normally must first pass through lipid bilayers in the intestinal epithelium and must be hydrophobic enough (high LogP values), but not too hydrophobic since the drug must be previously dissolved and, once it is in the bilayer, it should not exit.[182] On the other hand, in terms of pharmacodynamic profile hydrophobic drugs tend to be more toxic because, in general, they are retained for longer periods and have a wider distribution within the body.[182] Christopher Lipinski developed the "Rule of five" and endorsed that a potential drug might demonstrate intermediate LogP values (between 2 and 5).[184]

During investigations aiming at the design, synthesis and evaluation of novel quinolones with potential activity against malaria and tuberculosis it was demonstrated from preliminary animal studies that, in general, ClogP needed to be reduced and aqueous solubility needed to be enhanced in order to administer the drug in a suitable vehicle without the need for a pro-drug approach.[3,4,64] Several strategies have been adopted to reduce ClogP, to improve aqueous solubility and to allow the possibility of salt formation (synthesis of pro-drugs), achieving an improvement of the solubility and drug delivery of the quinolone-core derivatives. Some modifications of the side chain of the quinolone core, for example by incorporating heterocyclic groups, such as pyridine and piperazine moieties (at position 7 for antimalarial drugs or at position 2 for antituberculotic drugs), enabled the transformation of highly insoluble compounds into candidates with excellent drug-like properties.[3,4,64,185]

Beyond the modification of the side chain of the quinolone core to maximize solubility and activity, the use of pro-drug approaches has also been examined by different authors. For example, a pharmacokinetic study has shown that compound **MTC420** (Figure 27), with potential interest against tuberculosis, exhibited limited solubility, since its absorption did not increase linearly with doses, from 10 to 50 mg/kg. However, its acetate pro-drug (Figure 27) demonstrated a significant increase in overall exposure (increased AUC, Cmax and bioavailability), at both the 10 and 50 mg/kg doses.[64]

Regarding the development of new quinolone-based antimalarial candidates, recent results demonstrated that compound **23** (Figure 28) and its phosphate and morpholine pro-drugs, **24** and **25**, are effective at clearing plasmodial infections. Compound **23** posed solubility problems using the standard suspension vehicle (SSV) for the drug-administration, resulting in a reduced parasite clearance, and thus the compound had to be dosed as a suspension. By using 5% DMSO and 5% EtOH in tetraglycol (DET) as vehicle, **23** was fully dissolved and 100% parasite clearance could then be achieved at 20 mg/kg. However, when using the salt formulation, based on the phosphate or the morpholine pro-drug **24** and **25 (**Figure 28), 100% parasite clearance was observed with SSV. Measured solubility values confirmed that the

incorporation of the heterocyclic ring improves solubility at low pH and also that the solubility is enhanced when the compounds are formulated as the phosphate salt.[4]

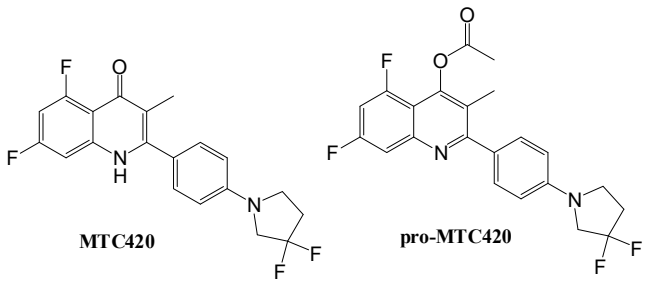


Figure 27. Structural representation of MTC420 and of its acetate pro-drug, studied by Hong *et al.*.

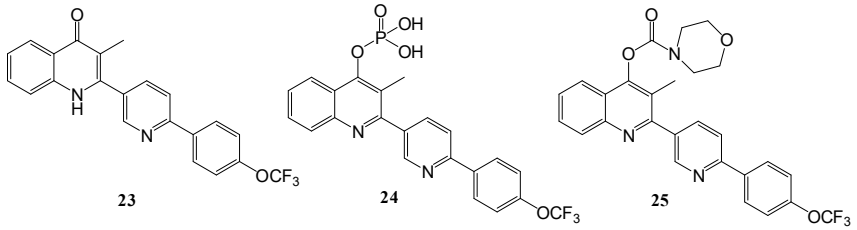


Figure 28. Structural representation of a quinolone-based antimalarial with poor solubility **23**, and of its soluble phosphate and morpholine pro-drug **24** and **25**.

The problematics of solubility also apply to fluoroquinolone antibiotics, which are known for their excellent activity against many difficult-to-treat bacteria.[7,8] Differences in activity observed for commercially available quinolones may result from subtle differences in substituents, that translate into differences in absorption and tissue penetration. Pharmacological activity requires the interaction between the drug and its target and, hence, antimicrobial activity depends on the transportation of the drugs across biological membranes (bacterial or gastrointestinal membranes). In general, it is recognized that more lipophilic compounds show better ability to cross the lipid membrane barriers and hence increased absorption.[186]

Edoka and Okeri determined the thermodynamic parameters of solubility for three fluoroquinolone antibiotics (ciprofloxacin, ofloxacin and norfloxacin) in 1-butanol and investigated their correlation with the transfer processes (absorption and penetration of bacterial cell wall). Simple solvent systems (aqueous-organic) have been used to simulate biomembranes, since they simulate the aqueous-lipid barriers found in the body. 1-Octanol and 1-butanol are, in most cases, the organic solvents of choice in these studies.[187] The results have shown that the solubility of the fluoroquinolones investigated was lower in the organic medium (1-butanol) than in the aqueous buffer, at the thermodynamic temperature of 25 ºC, increasing with the increase in temperature. The variation in standard free energy of solubilization for each of the three fluoroquinolones was higher in 1-butanol than in the buffered aqueous medium, meaning that more energy or work is needed to solubilize fluoroquinolones in 1-butanol, compared to the aqueous medium. The spontaneity of the solubilization as well as the relative size of the standard change in enthalpies and entropies was dependent on temperature. From the results obtained for all parameters analyzed, norfloxacin demonstrated the best performance, showing to be the most soluble in 1-butanol, which is related to the much lower standard change in free energy of solubilization for norfloxacin in 1-butanol.[188]

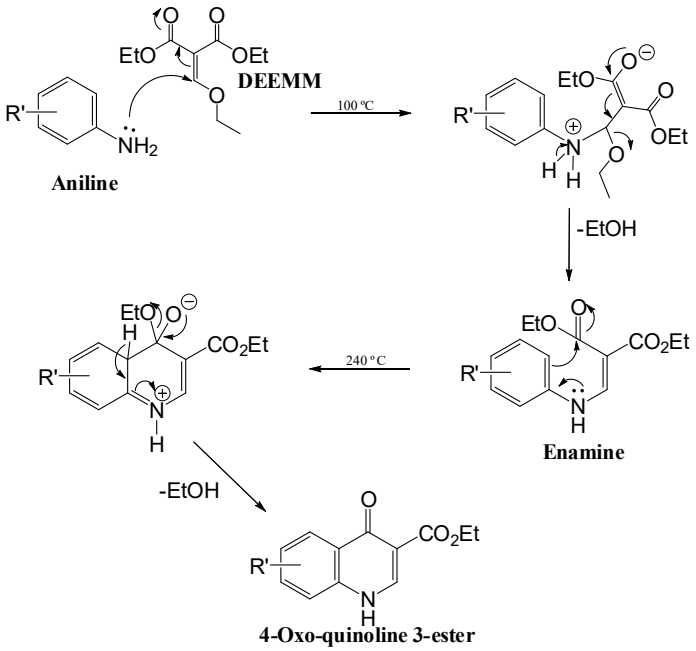
4.6. Synthesis inherent problems

The synthesis of 4-oxo-quinolines attracts special attention because more synthesis-related problems have been raised for this chemotype than for other quinoline sub-classes. The Gould-Jacobs reaction (an

adaptation of the Conrad-Limpach synthesis) is one of the most used synthetic strategies for the preparation of novel 4-oxo-quinolines. In this thermally driven cyclisation methodology it is proposed that the phenyl ring acts as nucleophile, attacking the carbon of the ester carbonyl group.[134] However, some criticisms were raised regarding the Gould-Jacobs methodology, mostly related to the high temperature required for cyclisation (above 225 °C), which may cause thermal degradation of susceptible groups (thermolysis) and lead to side reactions.[13] In fact, the yields for conversion of the enamine derivatives into the quinolone scaffolds, following the thermally driven Gould-Jacobs intramolecular cyclisation methodology, may vary from very low to excellent and for some of the enamine derivatives no product is recovered. Also, the cyclisation was found to be concentration dependent and the high boiling point of the solvent (Dowtherm A) renders its total extraction or separation from the desired organic products a difficult task. In addition, most quinolones exhibit low solubility, leading to difficulties in extraction and purification and this procedure may lead to side products, some of them resulting from structural isomerism, as detailed in the following sections.[35]

4.6.1. Structural isomerism in quinolone synthesis

According to the mechanism proposed for quinolone synthesis (Scheme 20), in the thermally driven cyclisation step the aromatic ring of the enamine ester acts as nucleophile, attacking the carbon of the ester carbonyl group and affording 4-oxo-quinoline 3-esters.[134]

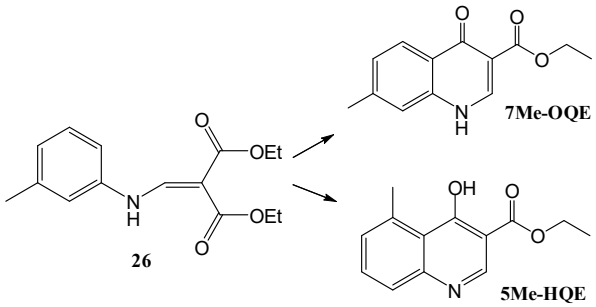


Scheme 20. Schematic representation of the mechanism proposed for the Gould-Jacobs synthesis of 4-oxo-quinoline 3-esters.

However, this path may result in a cyclisation involving both *ortho* carbons adjacent to the NH group of the enamine derivative, leading to an isomeric mixture of quinolones bearing different substitution patterns on the carbocyclic ring. This constraint was evident throughout the research conducted by Horta *et al.*, affecting compounds with diverse substituent groups, although with different impact.[35]

Alternative methodologies were developed to circumvent the liabilities of the Gould-Jacobs cyclisation, one of them involving, in one pot, cyclisation of an enamine and chlorination at position 4 of the quinoline core, mediated by phosphoryl chloride.[190] However, this strategy does not prevent the formation of

products with different substitution patterns or isomeric mixtures.[35] For instance, the cyclisation of enamine **26** (Scheme 21) was conducted in Dowtherm A (thermal cyclisation) and using $POCl_3$. It is worth noticing that these two procedures afforded quinolines with different patterns of aromatic substitution, resulting from cyclisation on both *ortho* carbons of the enamine precursor. The thermal cyclisation afforded a quinolone with the substituent (methyl group) at 7-position (**7Me-OQE**), while the cyclisation mediated by $POCl_3$ led to an hydroxyquinoline substituted at 5-position (**5Me-HQE**).[35] Thus, in addition to the difficulties in controlling regioselectivity, oxo-quinoline/hydroxy-quinoline tautomerism may be observed.[34]



Scheme 21. Synthetic approach to ethyl 4-oxo-7-methylquinoline-3-carboxylate **7Me-OQE** and to ethyl 4-hydroxy-5-methylquinoline-3-carboxylate **5Me-HQE**

4.6.2. Oxo-quinoline/hydroxy-quinoline tautomerism

Conventional quinolones (*e.g.* nalidixic acid, ciprofloxacin and norfloxacin) that are widely used in medicine as antibacterial agents are *N*-alkyl substituted, the *N*-substituent hindering the possibility of tautomerism.[7]

However, during the development of quinolones with antimalarial activity, structure-activity relationship studies on the quinolone 3-ester chemotype and docking studies performed *in silico* at the yeast Q_o site of the bc_1 protein complex of *P. falciparum* indicated that the 4-oxo-quinoline and the ethyl ester moieties are relevant for activity. These docking studies indicated that the 4-oxo-quinoline moiety is oriented to enable formation of H-bond between the quinolone carbonyl and a protonated imidazole N atom of His181, while a second H-bond (water mediated) is predicted between the carboxyl group of the Glu272 and the quinolone N-H. Thus, both the 4-oxo substituent and the N-H group appear to be important to the antimalarial activity of quinolone 3-esters.[6] Hence, the possibility of tautomerism between 4-oxo-quinoline and 4-hydroxy-quinoline forms should not be neglected.

As reported in the previous section, the research performed by Horta *et al.* demonstrated the possibility of a quinolone-hydroxyquinoline tautomerism in quinolone 3-esters. This fact may translate to alterations of chemical and physical properties, with consequences for pharmacokinetic and pharmacodynamic profiles, affecting activity and/or mode of action, resulting from alterations in drug-target interactions. Thus, the group investigated the tautomer and conformer preferences in quinolone 3-esters, using ethyl 4-oxo-7-methylquinoline-3-carboxylate **7Me-OQE** and ethyl 4-hydroxy-5-methylquinoline-3-carboxylate **5Me-HQE** as model compounds. **7Me-OQE** was obtained by thermal cyclisation of **26** but the corresponding 4-hydroxy-quinoline tautomer **7Me-HQE** was not isolated. However, $POCl_3$ mediated cyclisation of **26** afforded 4-hydroxy-quinoline **5Me-HQE** (Figure 29).[35]

During structural studies of representative quinolone 3-esters performed by Horta *et al.*, the conformational and tautomeric preferences of monomeric **5Me-HQE** and **7Me-OQE** were theoretically investigated and the possibility of quinolone-hydroxyquinoline equilibria was evaluated. Calculations showed preference for the hydroxy-quinoline form in both compounds, with a difference between the lowest energy hydroxy-quinoline and oxo-quinoline forms of around 27 and 38 $kJ.mol^{-1}$, for **5Me-HQE** and **7Me-OQE**, respectively. The lower energy of the hydroxy-quinoline form was related to the presence of an intramolecular H-bond interaction, O-H···O(ester carbonyl), and to the highest aromatic character exhibited by the heterocyclic ring of quinoline in the enol form.[35]

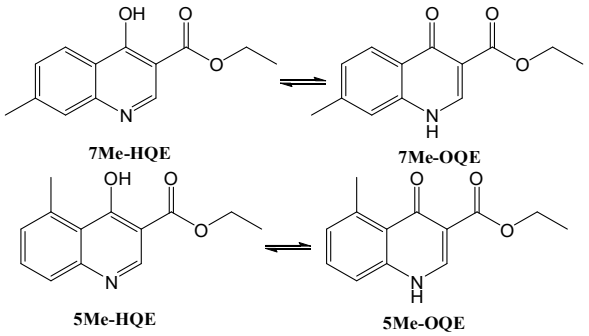


Figure 29. Tautomers of ethyl 7-methyl-4-oxo-quinoline 3-carboxylate **7Me-OQE** and **7Me-HQE** and of ethyl 5-methyl-4-oxo-quinoline-3-carboxylate **5Me-OQE** and **5Me-HQE**, studied by Horta *et al.*.

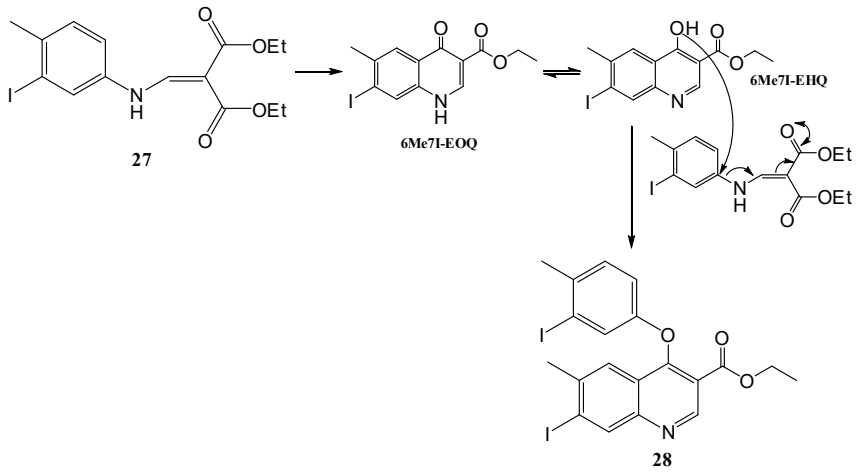
The investigation of the monomeric structure using matrix isolation has shown that the hydroxy-quinoline tautomeric form of the compound does not tautomerize upon sublimation. On the contrary, when the solid 4-oxo-quinoline (keto) form was used as starting material for the matrix isolation experiments, extensive tautomerization was observed, resulting in the sole observation of the 4-hydroxy-quinoline tautomeric form.[35]

While the substitution pattern on the carbocyclic part of the quinolone bicyclic structure (7- or 5-substituted derivatives) does not appear to interfere with the lipophilicity/solubility profile, tautomerism clearly impacts. The keto forms showed a lower CLogP than the corresponding hydroxy forms. Thus, the keto forms present a higher aqueous solubility, but their reduced lipophilicity decreases the passage through biological membranes. So, the CLogP values exhibited by the hydroxy forms appear more adequate from a bioavailability viewpoint, although their pharmacodynamic profile as inhibitors of the *P. falciparum* bc_1 complex acting at the Q_o site is expected to be less favourable, in view of the results from docking studies. However, the activity results showed that the 4-oxo-quinoline derivatives were only slightly more active than their 4-hydroxy-quinoline analogues. These results raise the hypothesis of quinolone binding to another site (for example to the Q_i site) and, at the same time, given the low CLogP value for 4-oxo-quinoline compared to the 4-hydroxy-quinoline, a better pharmacodynamic performance may be offset by the lower bioavailability.[35] Additional docking studies should be undertaken with this hydroxy-quinoline class, using relevant enzyme targets.

As previously described, solubility also affects the efficacy of synthetic strategies. The synthesis of chloroquinoline derivatives as intermediates may circumvent this problem, since they are more easily solubilized and isolated and may be subsequently converted into the corresponding quinolones.[34,36,190] However, some problems may arise in $POCl_3$ mediated cyclisation, such as the formation of unexpected products. For example, attempts to synthesize a 4-oxo-quinoline derivative from compound **27**, following the $POCl_3$ mediated strategy, led to formation of 4-aryloxy-quinoline 3-ester **28**. The isolation of this compound confirms the impact of quinolone/hydroxyquinoline tautomerism on the fate of the synthetic strategy. As depicted in Scheme 22, the authors proposed the initial formation of 4-oxo-quinoline 3-ester **6Me7I-EOQ** which may then undergo tautomerization to the corresponding 4-hydroxy-quinoline 3-ester **6Me7I-EHQ**. Once formed, the hydroxy-quinoline tautomer may act as nucleophile (through its 4-hydroxyl group), reacting with the enamine precursor and subsequently affording the 4-aryloxyquinoline.[36]

5. Conclusions

While the relevance of quinolones in medicinal chemistry calls for more reliable synthetic methodologies to access the class, optimization of the synthetic procedures clearly demands a better understanding of the reactions involved, including a detailed structural analysis of reactants, intermediate compounds, by-products formed and desired products. In addition, a deeper knowledge of the structure of quinolones is also important to better predict or interpret biological activity.



Scheme 22. Schematic representation of the mechanism proposed for the synthesis of 7-iodo-4-(3-iodo-4-methylphenoxy)-6-methyl-quinoline-3-carboxylate **28**.

Acknowledgements

The authors gratefully acknowledge 'Fundação para a Ciência e Tecnologia' (FCT) for projects UID/Multi/04326/2018 (Centre of Marine Sciences - CCMAR) and PTDC/MAR-BIO/4132/2014, and for grants SFRH/BD/81821/2011 (Pedro Horta) and SFRH/BD/140249/2018 (Alina Secrieru). 'Centro de Química de Coimbra' (Departamento de Química, Universidade de Coimbra),'Department of Chemistry' (University of Liverpool) and Global Health and Tropical Medicine (New University of Lisbon) are gratefully acknowledged for the collaboration and availability to, jointly, contribute to enlarge the frontiers of knowledge.

References

1. O'Donnell, J. A.; Gelone, S. P. *Infect. Dis. Clin. North Am.* **2000**, *14*, 489-513.
2. R. Solomon, V.; Lee, H. *Curr. Med. Chem.* **2011**, *18*, 1488-1508.
3. Pidathala, C.; Amewu, R.; Pacorel, B.; Nixon, G. L.; Gibbons, P.; Hong, W. D.; Leung, S. C.; Berry, N. G.; Sharma, R.; Stocks, P. A.; Srivastava, A.; Shone, A. E.; Charoensutthivarakul, S.; Taylor, L.; Berger, O.; Mbekeani, A.; Hill, A.; Fisher, N. E.; Warman, A. J.; Biagini, G. A.; Ward, S. A.; O'Neill, P. M. *J. Med. Chem.* **2012**, *55*, 1831-1843.
4. Leung, S. C.; Gibbons, P.; Amewu, R.; Nixon, G. L.; Pidathala, C.; Hong, W. D.; Pacorel, B.; Berry, N. G.; Sharma, R.; Stocks, P. A.; Srivastava, A.; Shone, A. E.; Charoensutthivarakul, S.; Taylor, L.; Berger, O.; Mbekeani, A.; Hill, A.; Fisher, N. E.; Warman, A. J.; Biagini, G. A.; Ward, S. A.; O'Neill, P. M. *J. Med. Chem.* **2012**, *55*, 1844-1857.
5. Biagini, G. A.; Fisher, N.; Shone, A. E.; Mubaraki, M. A.; Srivastava, A.; Hill, A.; Antoine, T.; Warman, A. J.; Davies, J.; Pidathala, C.; Amewu, R. K.; Leung, S. C.; Sharma, R.; Gibbons, P.; Hong, D. W.; Pacorel, B.; Lawrenson, A. S.; Charoensutthivarakul, S.; Taylor, L.; Berger, O.; Mbekeani, A.; Stocks, P. A.; Nixon, G. L.; Chadwick, J.; Hemingway, J.; Delves, M. J.; Sinden, R. E.; Zeeman, A.-M.; Kocken, C. H. M.; Berry, N. G.; O'Neill, P. M.; Ward, S. A. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 8298-8303.
6. Cowley, R.; Leung, S.; Fisher, N.; Al-Helal, M.; Berry, N. G.; Lawrenson, A. S.; Sharma, R.; Shone, A. E.; Ward, S. A.; Biagini, G. A.; O'Neill, P. M. *Medchemcomm* **2012**, *3*, 39-44.
7. Sissi, C.; Palumbo, M. *Curr. Med. Chem. Anticancer. Agents* **2003**, *3*, 439-450.
8. Kim, O. K.; Ohemeng, K.; Barrett, J. F. *Expert Opin. Investig. Drugs* **2001**, *10*, 199-212.
9. Kathrotiya, H. G.; Patel, M. P. *Eur. J. Med. Chem.* **2013**, *63*, 675-684.
10. Tedesco, R.; Shaw, A. N.; Bambal, R.; Chai, D.; Concha, N. O.; Darcy, M. G.; Dhanak, D.; Fitch, D. M.; Gates, A.; Gerhardt, W. G.; Halegoua, D. L.; Han, C.; Hofmann, G. A.; Johnston, V. K.; Kaura, A.

C.; Liu, N.; Keenan, R. M.; Lin-Goerke, J.; Sarisky, R. T.; Wiggall, K. J.; Zimmerman, M. N.; Duffy, K. J. *J. Med. Chem.* **2006**, *49*, 971-983.
11. Kumar, D. V; Rai, R.; Brameld, K. A.; Somoza, J. R.; Rajagopalan, R.; Janc, J. W.; Xia, Y. M.; Ton, T. L.; Shaghafi, M. B.; Hu, H.; Lehoux, I.; To, N.; Young, W. B.; Green, M. J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 82-87.
12. Serrao, E.; Debnath, B.; Otake, H.; Kuang, Y.; Christ, F.; Debyser, Z.; Neamati, N. *J. Med. Chem.* **2013**, *56*, 2311-2322.
13. Dorow, R. L.; Herrinton, P. M.; Hohler, R. A.; Maloney, M. T.; Mauragis, M. A.; McGhee, W. E.; Moeslein, J. A.; Strohbach, J. W.; Veley, M. F. *Org. Process Res. Dev.* **2006**, *10*, 493-499.
14. Sultana, N.; Naz, A.; Khan, B.; Arayne, M. S.; Mesaik, M. A. *Med. Chem. Res.* **2009**, *19*, 1210-1221.
15. Kumar, A.; Fernandes, J.; Kumar, P. *Int. J. Pharm. Sci. Drug Res.* **2014**, *6*, 124-127.
16. Nakamura, S.; Kozuka, M.; Bastow, K. F.; Tokuda, H.; Nishino, H.; Suzuki, M.; Tatsuzaki, J.; Morris Natschke, S. L.; Kuo, S.-C.; Lee, K.-H. *Bioorg. Med. Chem.* **2005**, *13*, 4396-4401.
17. Murray, M. C.; Perkins, M. E. In *Annual Reports in Medicinal Chemistry* **1996**; pp 141-150.
18. Meshnick, S. R.; Dobson, M. J. In *Antimalarial Chemotherapy*; Philip J. Rosenthal MD, Ed.; Humana Press, **2001**; pp 15-25.
19. Hofheinz, W.; Merkli, B. In *Antimalarial Drugs II: Current Antimalarials and New Drug Developments*; Peters, W., Rogers, W. H. G., Eds.; Springer Berlin Heidelberg: London, **1984**; pp 61-81.
20. O 'Neill, P. M.; Bray, P. G.; Hawley, S. R.; Ward, S. A.; Park, B. K. *Pharmacol. Ther.* **1998**, *77*, 29-58.
21. Leventhal, R.; Cheadle, R. F. *Medical Parasitology: A Self-instructional Text*, 6th ed.; F.A. Davis Company, **2012**, 98-103.
22. Brunton, L. L.; Parker, K. L. *Goodman and Gilman Manual of Pharmacology and Therapeutics*, 12nd ed.; McGraw Hill Professional, **2008**, 661-680.
23. Rosenthal, P. J. In *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery*; Rosenthal, P. J. Eds.; Humana Press, **2001**; pp 87-106.
24. Shetty, N.; Tang, J. W.; Andrews, J. *Infectious Disease: Pathogenesis, Prevention and Case Studies*; John Wiley & Sons, **2009**, 458-475.
25. Beteck, R. M.; Smit, F. J.; Haynes, R. K.; N'Da, D. D. *Malar. J.* **2014**, *13*, 339-348.
26. Salzer, W.; Timmler, H.; Andersag, H. *Chem. Ber.* **1948**, *81*, 12-19.
27. Winter, R. W.; Kelly, J. X.; Smilkstein, M. J.; Dodean, R.; Hinrichs, D.; Riscoe, M. K. *Exp. Parasitol.* **2008**, *118*, 487-497.
28. Casey, A. C. *J. Med. Chem.* **1974**, *17*, 255-256.
29. Gaillard, T.; Madamet, M.; Tsombeng, F. F.; Dormoi, J.; Pradines, B. *Malar. J.* **2016**, *15*, 556-565.
30. Ryley, J. F.; Peters, W. *Ann. Trop. Med. Parasitol.* **1970**, *64*, 209-222.
31. Fisher, N.; Bray, P. G.; Ward, S. A.; Biagini, G. A. *Trends Parasitol.* **2007**, *23*, 305-310.
32. Nixon, G. L.; Pidathala, C.; Shone, A. E.; Antoine, T.; Fisher, N.; O'Neill, P. M.; Ward, S. A.; Biagini, G. A. *Future Med. Chem.* **2013**, *5*, 1573-1591.
33. Fisher, N.; Meunier, B. *FEMS Yeast Res.* **2008**, *8*, 183-192.
34. Horta, P. C.; Henriques, M. S. C.; Kuş, N.; Paixão, J. A.; O'Neill, P. M.; Cristiano, M. L. S.; Fausto, R. *Tetrahedron* **2015**, *71*, 7583-7592.
35. Horta, P.; Kuş, N.; Henriques, M. S. C.; Paixão, J. A.; Coelho, L.; Nogueira, F.; O'Neill, P. M.; Fausto, R.; Cristiano, M. L. S. *J. Org. Chem.* **2015**, *80*, 12244-12257.
36. Horta, P.; Henriques, M. S. C.; Brás, E. M.; Murtinheira, F.; Nogueira, F.; O'Neill, P. M.; Paixão, J. A.; Fausto, R.; Cristiano, M. L. S. *Pure Appl. Chem.* **2017**, *89*, 765-780.
37. Capper, M. J.; O'Neill, P. M.; Fisher, N.; Strange, R. W.; Moss, D.; Ward, S. A.; Berry, N. G.; Lawrenson, A. S.; Hasnain, S. S.; Biagini, G. A.; Antonyuk, S. V. *Proc. Natl. Acad. Sci.* **2015**, *112*, 755-760.
38. Winter, R.; Kelly, J. X.; Smilkstein, M. J.; Hinrichs, D.; Koop, D. R.; Riscoe, M. K. *Exp. Parasitol.* **2011**, *127*, 545-551.
39. Cross, R. M.; Monastyrskyi, A.; Mutka, T. S.; Burrows, J. N.; Kyle, D. E.; Manetsch, R. *J. Med.*

Chem. **2010**, *53*, 7076-7094.
40. Nilsen, A.; LaCrue, A. N.; White, K. L.; Forquer, I. P.; Cross, R. M.; Marfurt, J.; Mather, M. W.; Delves, M. J.; Shackleford, D. M.; Saenz, F. E.; Morrisey, J. M.; Steuten, J.; Mutka, T.; Li, Y.; Wirjanata, G.; Ryan, E.; Duffy, S.; Kelly, J. X.; Sebayang, B. F.; Zeeman, A.-M.; Noviyanti, R.; Sinden, R. E.; Kocken, C. H. M.; Price, R. N.; Avery, V. M.; Angulo-Barturen, I.; Jiménez-Díaz, M. B.; Ferrer, S.; Herreros, E.; Sanz, L. M.; Gamo, F.-J.; Bathurst, I.; Burrows, J. N.; Siegl, P.; Guy, R. K.; Winter, R. W.; Vaidya, A. B.; Charman, S. A.; Kyle, D. E.; Manetsch, R.; Riscoe, M. K. *Sci. Transl. Med.* **2013**, *5*, 177ra37.
41. da Cruz, F. P.; Martin, C.; Buchholz, K.; Lafuente-Monasterio, M. J.; Rodrigues, T.; Sönnichsen, B.; Moreira, R.; Gamo, F.-J.; Marti, M.; Mota, M. M.; Hannus, M.; Prudêncio, M. *J. Infect. Dis.* **2012**, *205*, 1278-1286.
42. Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, R. P. *J. Med. Pharm. Chem.* **1962**, *5*, 1063-1065.
43. Bisacchi, G. S. *J. Med. Chem.* **2015**, *58*, 4874-4882.
44. Bolon, M. K. *Med. Clin. North Am.* **2011**, *95*, 793-817.
45. Naeem, A.; Badshah, S.; Muska, M.; Ahmad, N.; Khan, K. *Molecules* **2016**, *21*, 268-286.
46. Andriole, V. T. *Clin. Infect. Dis.* **2005**, *41*, S113–S119.
47. Stein, G. E. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* **1988**, *8*, 301-314.
48. Tillotson, G. S. *J. Med. Microbiol.* **1996**, *44*, 320-324.
49. Emmerson, A. M. *J. Antimicrob. Chemother.* **2003**, *51*, 13-20.
50. Divo, A. A.; Sartorelli, A. C.; Patton, C. L.; Bia, F. J. *Antimicrob. Agents Chemother.* **1988**, *32*, 1182-1186.
51. Watt, G.; Shanks, G. D.; Edstein, M. D.; Pavanand, K.; Webster, H. K.; Wechgritaya, S. *J. Infect. Dis.* **1991**, *164*, 602-604.
52. McClean, K. L.; Hitchman, D.; Shafran, S. D. *J. Infect. Dis.* **1992**, *165*, 904-907.
53. Ciceri, K. *WHO policy on collaborative TB/HIV activities, Guidelines for national programmes and other stakeholders*; WHO Eds.; **2012**, 1-36.
54. Ginsberg, A. M.; Lampis, G.; Fioravanti, R.; Biava, M.; Poretta, G. C.; Zanetti, S.; Al., E.; Nacy, C. A. *Tuberculosis* **2010**, *90*, 162-167.
55. van den Boogaard, J.; Kibiki, G. S.; Kisanga, E. R.; Boeree, M. J.; Aarnoutse, R. E. *Antimicrob. Agents Chemother.* **2009**, *53*, 849-862.
56. Ballell, L.; Bates, R. H.; Young, R. J.; Alvarez-Gomez, D.; Alvarez-Ruiz, E.; Barroso, V.; Blanco, D.; Crespo, B.; Escribano, J.; González, R.; Lozano, S.; Huss, S.; Santos-Villarejo, A.; Martín-Plaza, J. J.; Mendoza, A.; Rebollo-Lopez, M. J.; Remuiñan-Blanco, M.; Lavandera, J. L.; Pérez-Herran, E.; Gamo-Benito, F. J.; García-Bustos, J. F.; Barros, D.; Castro, J. P.; Cammack, N. *Chem. Med. Chem.* **2013**, *8*, 313-321.
57. Facchinetti, V.; Gomes, C. R.; de Souza, M. V.; Vasconcelos, T. R. *Mini Rev. Med. Chem.* **2012**, *12*, 866-874.
58. Gillespie, S. H.; Crook, A. M.; McHugh, T. D.; Mendel, C. M.; Meredith, S. K.; Murray, S. R.; Pappas, F.; Phillips, P. P. J.; Nunn, A. J. *N. Engl. J. Med.* **2014**, *371*, 1577-1587.
59. Singh, S.; Kaur, G.; Mangla, V.; Gupta, M. K. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 492-504.
60. Senthilkumar, P.; Dinakaran, M.; Banerjee, D.; Devakaram, R. V.; Yogeeswari, P.; China, A.; Nagaraja, V.; Sriram, D. *Bioorg. Med. Chem.* **2008**, *16*, 2558-2569.
61. Dinakaran, M.; Senthilkumar, P.; Yogeeswari, P.; China, A.; Nagaraja, V.; Sriram, D. *Bioorg. Med. Chem.* **2008**, *16*, 3408-3418.
62. Sriram, D.; Aubry, A.; Yogeeswari, P.; Fisher, L. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2982-2985.
63. Talath, S.; Gadad, A. K. *Eur. J. Med. Chem.* **2006**, *41*, 918-924.
64. Hong, W. D.; Gibbons, P. D.; Leung, S. C.; Amewu, R.; Stocks, P. A.; Stachulski, A. V.; Horta, P. C.; Cristiano, M. L. S.; Shone, A. E.; Moss, D.; Ardrey, A.; Sharma, R.; Warman, A. J.; Bedingfield, P. T. P.; Fisher, N. E.; Aljayyoussi, G.; Mead, S.; Caws, M.; Berry, N. G.; Ward, S. A.; Biagini, G. A.; O'Neill, P. M.; Nixon, G. L. *J. Med. Chem.* **2017**, *60*, 3703-3726.
65. Warman, A. J.; Rito, T. S.; Fisher, N. E.; Moss, D. M.; Berry, N. G.; O'Neill, P. M.; Ward, S. A.;

Biagini, G. A. *J. Antimicrob. Chemother.* **2013**, *68*, 869-880.
66. Mdluli, K.; Ma, Z. *Infect. Disord. Drug Targets* **2007**, *7*, 159-168.
67. National Cancer Institute, *What Is Cancer?;* National Cancer Institute Eds,; **2015**.
68. Mugnaini, C.; Pasquini, S.; Corelli, F. *Curr. Med. Chem.* **2009**, *16*, 1746-1767.
69. Foroumadi, A.; Emami, S.; Rajabalian, S.; Badinloo, M.; Mohammadhosseini, N.; Shafiee, A. *Biomed. Pharmacother.* **2009**, *63*, 216-220.
70. You, Q.-D.; Li, Z.-Y.; Huang, C.-H.; Yang, Q.; Wang, X.-J.; Guo, Q.-L.; Chen, X.-G.; He, X.-G.; Li, T.-K.; Chern, J.-W. *J. Med. Chem.* **2009**, *52*, 5649-5661.
71. Yamashita, Y.; Ashizawa, T.; Morimoto, M.; Hosomi, J.; Nakano, H. *Cancer Res.* **1992**, *52*, 2818-2822.
72. Bisacchi, G. S.; Hale, M. R. *Curr. Med. Chem.* **2016**, *23*, 520-577.
73. Abbas, J. A.; Stuart, R. K. *Expert Opin. Investig. Drugs* **2012**, *21*, 1223-1233.
74. Tsuzuki, Y.; Tomita, K.; Shibamori, K.; Sato, Y.; Kashimoto, S.; Chiba, K. *J. Med. Chem.* **2004**, *47*, 2097-2109.
75. United Nations Programme on HIV/AIDS, *Global AIDS Update 2016*; WHO Eds.; **2016**, 1-16.
76. Li, Y.; Xuan, S.; Feng, Y.; Yan, A. *Drug Discovery Today*. **2015**, *20*, 435-449.
77. Dayam, R.; Al-Mawsawi, L. Q.; Zawahir, Z.; Witvrouw, M.; Debyser, Z.; Neamati, N. *J. Med. Chem.* **2008**, *51*, 1136-1144.
78. Shibagaki, Y.; Chow, S. A. *J. Biol. Chem.* **1997**, *272*, 8361-8369.
79. Ahmed, A.; Daneshtalab, M. *J. Pharm. Pharm. Sci.* **2012**, *15*, 52-72.
80. Serrao, E.; Odde, S.; Ramkumar, K.; Neamati, N. *Retrovirology* **2009**, *6*, 25-38.
81. Costi, R.; Métifiot, M.; Chung, S.; Cuzzucoli Crucitti, G.; Maddali, K.; Pescatori, L.; Messore, A.; Madia, V. N.; Pupo, G.; Scipione, L.; Tortorella, S.; Di Leva, F. S.; Cosconati, S.; Marinelli, L.; Novellino, E.; Le Grice, S. F. J.; Corona, A.; Pommier, Y.; Marchand, C.; Di Santo, R. *J. Med. Chem.* **2014**, *57*, 3223-3234.
82. Ellis, D.; Kuhen, K. L.; Anaclerio, B.; Wu, B.; Wolff, K.; Yin, H.; Bursulaya, B.; Caldwell, J.; Karanewsky, D.; He, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4246-4251.
83. Tabarrini, O.; Massari, S.; Cecchetti, V. *Future Med. Chem.* **2010**, *2*, 1161-1180.
84. Tabarrini, O.; Massari, S.; Daelemans, D.; Meschini, F.; Manfroni, G.; Bottega, L.; Gatto, B.; Palumbo, M.; Pannecouque, C.; Cecchetti, V. *Chem. Med. Chem.* **2010**, *5*, 1880-1892.
85. Neukam, K.; Macías, J.; Mira, J. A.; Pineda, J. A. *Expert Opin. Pharmacother.* **2009**, *10*, 417-433.
86. Nowotny, M. *EMBO Rep.* **2009**, *10*, 144-151.
87. Chen, Y. L.; Zacharias, J.; Vince, R.; Geraghty, R. J.; Wang, Z. *Bioorg. Med. Chem.* **2012**, *20*, 4790-4800.
88. WHO, *WHO guidelines for the treatment of genital herpes simplex virus*; WHO Eds.; **2016**, 1-56.
89. Lucero, B. D. A.; Gomes, C. R. B.; Frugulhetti, I. C. D. P. P.; Faro, L. V.; Alvarenga, L.; De Souza, M. C. B. V; De Souza, T. M. L.; Ferreira, V. F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1010-1013.
90. Musiol, R.; Serda, M.; Hensel-Bielowka, S.; Polanski, J. *Curr. Med. Chem.* **2010**, *17*, 1960-1973.
91. Wang, Y.; Damu, G. L. V.; Lv, J.-S.; Geng, R.-X.; Yang, D.-C.; Zhou, C.-H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5363-5366.
92. Zhang, L.; Kumar, K. V.; Geng, R.-X.; Zhou, C.-H. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3699-3705.
93. Sultana, N.; Arayne, M. S.; Gul, S.; Shamim, S. *J. Mol. Struct.* **2010**, *975*, 285-291.
94. Wu, C.; Sun, D. *Metab. Brain Dis.* **2015**, *30*, 367-379.
95. Kahnberg, P.; Howard, M. H.; Liljefors, T.; Nielsen, M.; Nielsen, E. Ø.; Sterner, O.; Pettersson, I. *J. Mol. Graph. Model.* **2004**, *23*, 253-261.
96. Lager, E.; Andersson, P.; Nilsson, J.; Pettersson, I.; Nielsen, E.; Nielsen, M.; Sterner, O.; Liljefors, T. *J. Med. Chem.* **2006**, *49*, 2526-2533.
97. Kocahan, S.; Doğan, Z. *Clin. Psychopharmacol. Neurosci.* **2017**, *15*, 1-8.
98. Chatterjee, A.; Cutler, S. J.; Doerksen, R. J.; Khan, I. A.; Williamson, J. S. *Bioorg. Med. Chem.* **2014**, *22*, 6409-6421.
99. Rom, S.; Persidsky, Y. *J. Neuroimmune Pharmacol.* **2013**, *8*, 608-620.
100. Rieder, S. A.; Chauhan, A.; Singh, U.; Nagarkatti, M.; Nagarkatti, P. *Immunobiology* **2010**, *215*, 598-

605.
101. Di Marzo, V. *Pharmacol. Res.* **2009**, *60*, 77-84.
102. Murikinati, S.; Jüttler, E.; Keinert, T.; Ridder, D. A.; Muhammad, S.; Waibler, Z.; Ledent, C.; Zimmer, A.; Kalinke, U.; Schwaninger, M. *FASEB J.* **2010**, *24*, 788-798.
103. Gebhart, G. F. *Am J. Physiol Gastrointest Liver Physiol.* **2000**, *278*, 834-838.
104. Fine, P. G.; Rosenfeld, M. J. *Rambam Maimonides Med. J.* **2013**, *4*, e0022.
105. Whiteside, G.; Lee, G.; Valenzano, K. *Curr. Med. Chem.* **2007**, *14*, 917-936.
106. Pasquini, S.; Ligresti, A.; Mugnaini, C.; Semeraro, T.; Cicione, L.; De Rosa, M.; Guida, F.; Luongo, L.; De Chiaro, M.; Cascio, M. G.; Bolognini, D.; Marini, P.; Pertwee, R.; Maione, S.; Marzo, V. Di; Corelli, F. *J. Med. Chem.* **2010**, *53*, 5915-5928.
107. Pasquini, S.; De Rosa, M.; Pedani, V.; Mugnaini, C.; Guida, F.; Luongo, L.; De Chiaro, M.; Maione, S.; Dragoni, S.; Frosini, M.; Ligresti, A.; Di Marzo, V.; Corelli, F. *J. Med. Chem.* **2011**, *54*, 5444-5453.
108. Mugnaini, C.; Brizzi, A.; Ligresti, A.; Allarà, M.; Lamponi, S.; Vacondio, F.; Silva, C.; Mor, M.; Di Marzo, V.; Corelli, F. *J. Med. Chem.* **2016**, *59*, 1052-1067.
109. Tiwari, A.; Singh, A. *Ovidius University Annals of Chemistry* **2013**, *24*, 5-12.
110. Dalhoff, A.; Shalit, I.; Hotta, K.; Al., E.; Ginsburg, G. *Lancet. Infect. Dis.* **2003**, *3*, 359-371.
111. Constantinescu, S. E.; Constantinescu, C. S. *Expert Rev. Clin. Pharmacol.* **2016**, *9*, 49-57.
112. Zilkha-Falb, R.; Gurevich, M.; Hayardeny, L.; Achiron, A. *J. Neuroimmunol.* **2015**, *283*, 11-16.
113. Zou, P.; Liu, L.; Zheng, L.; Liu, L.; Stoneman, R. E.; Cho, A.; Emery, A.; Gilbert, E. R.; Cheng, Z. *Cell Cycle* **2014**, *13*, 3759-3767.
114. Nagashima, T.; Shigematsu, N.; Maruki, R.; Urano, Y.; Tanaka, H.; Shimaya, A.; Shimokawa, T.; Shibasaki, M. *Mol. Pharmacol.* **2010**, *78*, 961-970.
115. Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny, J. M.; Moghal, N.; Lubkin, M.; Kim, Y. B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. *Mol. Cell. Biol.* **2000**, *20*, 5479-5489.
116. Zhi, Y.; Gao, L.-X.; Jin, Y.; Tang, C.-L.; Li, J.-Y.; Li, J.; Long, Y.-Q. *Bioorg. Med. Chem.* **2014**, *22*, 3670-3683.
117. Hillard, C. J. *Curr. Pharm. Des.* **2008**, *14*, 2347-2361.
118. Pacher, P.; Haskó, G. *Br. J. Pharmacol.* **2008**, *153*, 252-262.
119. Park, C. H.; Lee, J.; Jung, H. Y.; Kim, M. J.; Lim, S. H.; Yeo, H. T.; Choi, E. C.; Yoon, E. J.; Kim, K. W.; Cha, J. H.; Kim, S. H.; Chang, D. J.; Kwon, D. Y.; Li, F.; Suh, Y. G. *Bioorg. Med. Chem.* **2007**, *15*, 6517-6526.
120. Skraup, Z. H. *Monatshefte für Chemie Chemical Monthly* **1880**, *1*, 316-318.
121. Edinger, A. *Berichte der Dtsch. Chem. Gesellschaft* **1896**, *29*, 2456-2460.
122. Friedländer, P.; Gohring, C. F. *Berichte der Dtsch. Chem. Gesellschaft* **1883**, *16*, 1833-1839.
123. Doebner, O.; v. Miller, W. *Berichte der Dtsch. Chem. Gesellschaft* **1881**, *14*, 2812-2817.
124. Pfitzinger, W. *J. Prakt. Chemie* **1886**, *33*, 100.
125. Povarov, L. S. *Russ. Chem. Rev.* **1967**, *36*, 656-670.
126. Tois, J.; Vahermo, M.; Koskinen, A. *Tetrahedron Lett.* **2005**, *46*, 735-737.
127. Mitsos, C.; Zografos, A.; Igglessi-Markopoulou, O. *Chem. Pharm. Bull.* **2000**, *48*, 211-214.
128. Zhou, C.; Dubrovsky, A. V.; Larock, R. C. *J. Org. Chem.* **2006**, *71*, 1626-1632.
129. Camps, R. *Berichte Dtsch. Chem. Gesellschaft* **1899**, *32*, 3228-3234.
130. Hu, W.; Lin, J.-P.; Song, L.-R.; Long, Y.-Q. *Org. Lett.* **2015**, *17*, 1268-1271.
131. Stern, E.; Millet, R.; Depreux, P.; Hénichart, J.-P. *Tetrahedron Lett.* **2004**, *45*, 9257-9259.
132. Zhang, J.; Han, X.; Lu, X. *Synlett* **2015**, *26*, 1744-1748.
133. Conrad, M.; Limpach, L. *Berichte der Dtsch. Chem. Gesellschaft* **1887**, *20*, 944-948.
134. Gould, R. G.; Jacobs, W. A. *J. Am. Chem. Soc.* **1939**, *61*, 2890-2895.
135. Liu, L.; Lu, H.; Wang, H.; Yang, C.; Zhang, X.; Zhang-Negrerie, D.; Du, Y.; Zhao, K. *Org. Lett.* **2013**, *15*, 2906-2909.
136. Mai, W.-P.; Sun, G.-C.; Wang, J.-T.; Song, G.; Mao, P.; Yang, L.-R.; Yuan, J.-W.; Xiao, Y.-M.; Qu, L.-B. *J. Org. Chem.* **2014**, *79*, 8094-8102.

137. Boteva, A. A.; Krasnykh, O. P. *Chem. Heterocycl. Compd.* **2009**, *45*, 757-785.
138. Bernini, R.; Cacchi, S.; Fabrizi, G.; Sferrazza, A. *Synthesis* **2009**, *2009*, 1209-1219.
139. Wang, Y.; Liang, H.; Chen, C.; Wang, D.; Peng, J. *Synthesis* **2015**, *47*, 1851-1860.
140. Shao, J.; Huang, X.; Hong, X.; Liu, B.; Xu, B. *Synthesis* **2012**, *44*, 1798-1805.
141. Zhao, T.; Xu, B. *Org. Lett.* **2010**, *12*, 212-215.
142. Niementowski, S. *Berichte der Dtsch. Chem. Gesellschaft* **1894**, *27*, 1394-1403.
143. Åkerbladh, L.; Nordeman, P.; Wejdemar, M.; Odell, L. R.; Larhed, M. *J. Org. Chem.* **2015**, *80*, 1464-1471.
144. Wu, J.; Xiang, S.; Zeng, J.; Leow, M.; Liu, X.-W. *Org. Lett.* **2015**, *17*, 222-225.
145. Manikandan, R.; Jeganmohan, M. *Org. Lett.* **2014**, *16*, 3568-3571.
146. Huang, Y.-N.; Li, Y.-L.; Li, J.; Deng, J. *J. Org. Chem.* **2016**, *81*, 4645-4653.
147. Ishida, T.; Kikuchi, S.; Yamada, T. *Org. Lett.* **2013**, *15*, 3710-3713.
148. Jackson, M. A.; Schutze, G. E. *Comm. Infect. Dis.-Pediatr.* **2016**, *138*, 20162706-20162706.
149. Golomb, B. A.; Koslik, H. J.; Redd, A. J. *BMJ Case Rep.* **2015**, *2015*, bcr2015209821, 1-10.
150. Gough, A.; Barsoum, N. J.; Mitchell, L.; McGuire, E. J.; de la Iglesia, F. A. *Toxicol. Appl. Pharmacol.* **1979**, *51*, 177-187.
151. Patterson, D. R. *Am. J. Med.* **1991**, *91*, 35S-37S.
152. Sendzik, J.; Lode, H.; Stahlmann, R. *Int. J. Antimicrob. Agents* **2009**, *33*, 194-200.
153. Adefurin, A.; Sammons, H.; Jacqz-Aigrain, E.; Choonara, I. *Arch. Dis. Child.* **2011**, *96*, 874-880.
154. Noel, G. J.; Bradley, J. S.; Kauffman, R. E.; Duffy, C. M.; Gerbino, P. G.; Arguedas, A.; Bagchi, P.; Balis, D. A.; Blumer, J. L. *Pediatr. Infect. Dis. J.* **2007**, *26*, 879-891.
155. Noel, G. J.; Blumer, J. L.; Pichichero, M. E.; Hedrick, J. A.; Schwartz, R. H.; Balis, D. A.; Melkote, R.; Bagchi, P.; Arguedas, A. *Pediatr. Infect. Dis. J.* **2008**, *27*, 483-489.
156. Slimings, C.; Riley, T. V. *J. Antimicrob. Chemother.* **2014**, *69*, 881-891.
157. Briasoulis, A.; Agarwal, V.; Pierce, W. J. *Cardiology* **2011**, *120*, 103-110.
158. Gao, X.; Wen, X.; Esser, L.; Quinn, B.; Yu, L.; Yu, C.-A.; Xia, D. *Biochemistry* **2003**, *42*, 9067-9080.
159. Li, H.; Zhu, X.-L.; Yang, W.-C.; Yang, G.-F. *Chem. Biol. Drug Des.* **2014**, *83*, 71-80.
160. Berry, E. A.; Huang, L.-S.; Lee, D.-W.; Daldal, F.; Nagai, K.; Minagawa, N. *Biochim. Biophys. Acta* **2010**, *1797*, 360-370.
161. Biagini, G. A.; Viriyavejakul, P.; O'Neill, P. M.; Bray, P. G.; Ward, S. A. *Antimicrob. Agents Chemother.* **2006**, *50*, 1841-1851.
162. Barton, V.; Fisher, N.; Biagini, G. A.; Ward, S. A.; O'Neill, P. M. *Curr. Opin. Chem. Biol.* **2010**, *14*, 440-446.
163. Xiang, H.; McSurdy-Freed, J.; Moorthy, G. S.; Hugger, E.; Bambal, R.; Han, C.; Ferrer, S.; Gargallo, D.; Davis, C. B. *J. Pharm. Sci.* **2006**, *95*, 2657-2672.
164. Hooper, D. C.; Jacoby, G. A. *Ann. N. Y. Acad. Sci.* **2015**, *1354*, 12-31.
165. Raidt, L.; Idelevich, E. A.; Dübbers, A.; Küster, P.; Drevinek, P.; Peters, G.; Kahl, B. C. *Pediatr. Infect. Dis. J.* **2015**, *34*, 700-705.
166. Peters, J. M.; Chen, N.; Gatton, M.; Korsinczky, M.; Fowler, E. V; Manzetti, S.; Saul, A.; Cheng, Q. *Antimicrob. Agents Chemother.* **2002**, *46*, 2435-2441.
167. Fivelman, Q.; Butcher, G.; Adagu, I.; Warhurst, D.; Pasvol, G. *Malar. J.* **2002**, *1*, 1-4.
168. Berry, A.; Senescau, A.; Lelièvre, J.; Benoit-Vical, F.; Fabre, R.; Marchou, B.; Magnaval, J. F. *Trans. R. Soc. Trop. Med. Hyg.* **2006**, *100*, 986-988.
169. Kessl, J. J.; Meshnick, S. R.; Trumpower, B. L. *Trends Parasitol.* **2007**, *23*, 494-501.
170. Teixeira, C.; Vale, N.; Pérez, B.; Gomes, A.; Gomes, J. R. B.; Gomes, P. *Chem. Rev.* **2014**, *114*, 11164-11220.
171. Hammond, D. J.; Burchell, J. R.; Pudney, M. *Mol. Biochem. Parasitol.* **1985**, *14*, 97-109.
172. Garrelts, J. C.; Godley, P. J.; Peterie, J. D.; Gerlach, E. H.; Yakshe, C. C. *Antimicrob. Agents Chemother.* **1990**, *34*, 931-933.
173. Del Rosso, J. Q. *Dermatol. Clin.* **2009**, *27* (1), 91–94.
174. Neuhofel, A. L.; Wilton, J. H.; Victory, J. M.; Hejmanowsk, L. G.; Amsden, G. W. *J. Clin. Pharmacol.* **2002**, *42*, 461-466.

175. Wohlt, P. D.; Zheng, L.; Gunderson, S.; Balzar, S. A.; Johnson, B. D.; Fish, J. T. *Am. J. Heal. Pharm.* **2009**, *66*, 1458-1467.
176. Davis, R. L.; Quenzer, R. W.; Kelly, H. W.; Powell, J. R. *Ann. Pharmacother.* **1992**, *26*, 11-13.
177. Carrillo, J. A.; Benitez, J. *Clin. Pharmacokinet.* **2000**, *39*, 127-153.
178. Kinzigschippers, M.; Fuhr, U.; Zaigler, M.; Dammeyer, J.; Rusing, G.; Labedzki, A.; Bulitta, J.; Sorgel, F. *Clin. Pharmacol. Ther.* **1999**, *65*, 262-274.
179. Falagas, M. E.; Rafailidis, P. I.; Rosmarakis, E. S. *Int. J. Antimicrob. Agents* **2007**, *29*, 374-379.
180. Ishikawa, M.; Hashimoto, Y. *J. Med. Chem.* **2011**, *54*, 1539-1554.
181. Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Discov.* **2007**, *6*, 881-890.
182 Kubinyi, H. *Farmaco. [Sci.]* **1979**, *34*, 248-276.
183. Pliška, V.; Testa, B.; Waterbeemd, H. van de. *Lipophilicity in Drug Action and Toxicology*; John Wiley & Sons Ltd: New York, **1996**, 263-293.
184. Lipinski, C. A. *Drug Discov. Today. Technol.* **2004**, *1*, 337-341.
185. Zask, A.; Kaplan, J.; Verheijen, J. C.; Richard, D. J.; Curran, K.; Brooijmans, N.; Bennett, E. M.; Toral-Barza, L.; Hollander, I.; Ayral-Kaloustian, S.; Yu, K. *J. Med. Chem.* **2009**, *52*, 7942-7945.
186. Bazile, S.; Moreau, N.; Bouzard, D.; Essiz, M. *Antimicrob. Agents Chemother.* **1992**, *36*, 2622-2627.
187. Sangster, J. *Octanol-water partition coefficients : fundamentals and physical chemistry*; Wiley, **1997**; pp. 113-154.
188. Eboka, C. J.; Okeri, H. A. *J. Sci Pr. Pharm* **2014**, *1*, 37-40.
189. Willard, A. K.; Smith, R. L.; Cragoe, E. J. *J. Org. Chem.* **1981**, *46*, 3846-3852.