Nonclassical Biological Activities of Quinolone Derivatives

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ABSTRACT- Quinolones are considered as a big family of multi-faceted drugs; their chemical synthesis is flexible and can be easily adapted to prepare new congeners with rationally devised structures. This is shown by the description of many thousands of derivatives in the literature. Scientists could accurately describe their QSAR, which is essential for effective drug design. This also gave them the chance to discover new and unprecedented activities, which makes quinolones an endless source of hope and enables further development of new clinically useful drugs.

Quinolones are among the most common frameworks present in the bioactive molecules that have dominated the market for more than four decades. Since 1962, 4(1*H*)-quinolone-3-carboxylic acid derivatives are widely used as antibacterial agents. Quinolones have a broad and potent spectrum of activity and are also used as second-line drugs to treat tuberculosis (TB). Recently, quinolones have been reported to display "nonclassical" biological activities, such as antitumor, anti-HIV-1 integrase, anti- HCV-NS3 helicase and -NS5B-polymerase activities.

The present review focuses on the structural modifications responsible for the transformation of an antibacterial into an anticancer agent and/or an antiviral agent. Indeed, quinolones' antimicrobial action is distinguishable among antibacterial agents, because they target different type II topoisomerase enzymes. Many derivatives of this family show high activity against bacterial topoisomerases and eukaryotic topoisomerases, and are also toxic to cultured mammalian cells and *in vivo* tumor models. Moreover, quinolones have shown antiviral activity against HIV and HCV viruses. In this context the quinolones family of drugs seem to link three different biological activities (antibacterial, anticancer, and the antiviral profiles) and the review will also provide an insight into the different mechanisms responsible for these activities among different species.

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INTRODUCTION

Quinolones as "privileged building blocks" with simple and flexible synthetic routes allow the production of large libraries of bioactive molecules. Because of their diversity, drug-like properties and similarities to specific targets they are considered a central scaffold to build chemical libraries with promising bioactivity potential.

The first quinolone discovery, as many important discoveries, was a result of serendipity. Lesher *et al.* (1962) (1) discovered the first quinolone derivative as an impurity in the chemical manufacturing of a batch of antimalarial agent chloroquine. Since then more than 10,000 quinolone derivatives have been patented or published which explains the enormous progress that has been made in understanding the molecular mechanisms of action behind the different pharmacological actions of this privileged molecule.

Quinolones as a class of antibacterial agents have been known for over 40 years. Although considerable results in the research of new antibacterial quinolones have already been achieved, they are still a matter of study because of the continuous demand of novel compounds active against resistant strains of bacteria. Research efforts are mainly focused on obtaining new compounds active against very resistant bacterial strains or acting on the mechanisms of resistance (1-4).

Currently, fluoroquinolones are approved as second-line drugs by the WHO to treat TB but their use in MDR-TB is increasing due to the fact that they have a broad and potent spectrum of activity and can also be administered orally. Moreover, they have favorable pharmacokinetic profiles and good absorption, including excellent penetration into host macrophages.

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As of today, the potential of fluoroquinolones as first line drugs is still under investigation (5-7).

The chemistry of 4-Quinolones

The original method for the preparation of 4-quinolones relies on the Gould-Jacobs reaction between a substituted aniline and a dialkyl alkoxymethylenemalonate (8).

Lappin cyclization (9) results in cycloacylation on heating at a high temperature to provide the quinoline-4-one system as shown in **Scheme 1**. SN^2 alkylation at N-1 followed by ester hydrolysis affords the substituted 4(1*H*) - quinolone-3-carboxylic acid. The Gould-Jacobs approach allows only the introduction of primary alkyl groups, thus limiting the preparation of derivatives with more complex substituents at N-1. In this respect the Grohe- Heitzer cycloacylation expanded the synthetic possibilities of quinolones as depicted in **Scheme 2** (10).

In the Grohe-Heitzer synthesis, the active methylene in a β-ketoester is condensed under dehydrating conditions with an ortho ester resulting in the formation of the enol ether which is further subjected to an addition-elimination reaction using an appropriate primary amine. The product obtained is cyclised through aromatic nucleophilic displacement of a leaving group (typically Cl, F) at the *ortho* position with respect to the activating carbonyl group. Final hydrolysis of the ester function under either basic or acidic conditions gives the quinolone-3-carboxylic acid (11).

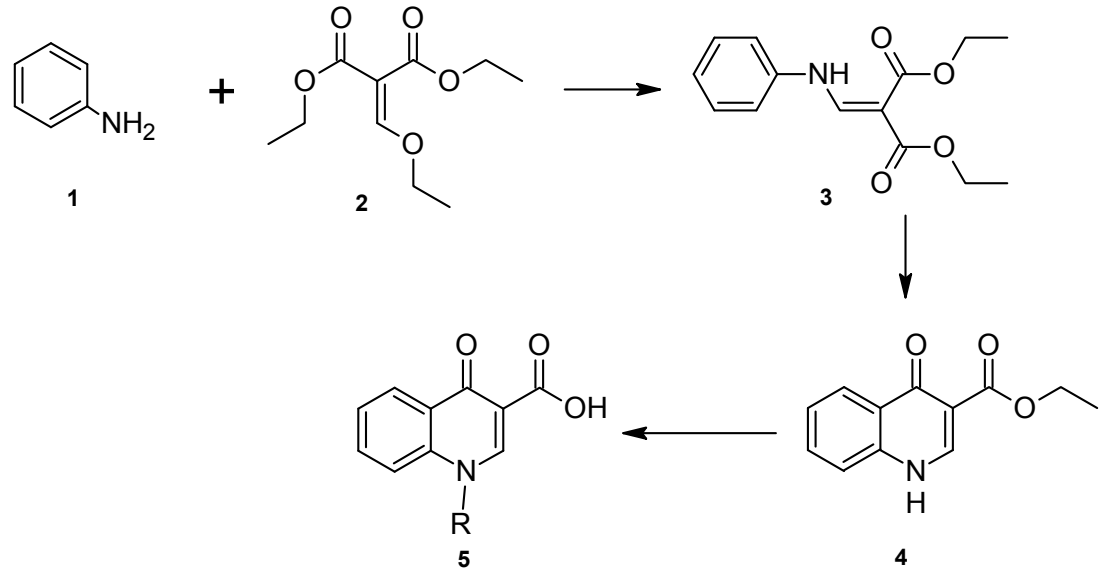
4(1*H*)-Quinolone-3-carboxylic acid derivatives containing a variety of substituents at different positions have also been prepared by palladium-catalyzed carbonylative heterocyclization (12). Palladium cyclization enables the synthesis of several 4(1*H*)-quinolone-3-carboxylic acid derivatives in 24-82% yields. The palladium-catalyzed cyclization has the potential to accommodate substituents at position 2 which in turn offers flexibility for the design of quinolinone derivatives as depicted in **Scheme 3**.

Biological targets of antibacterial quinolones

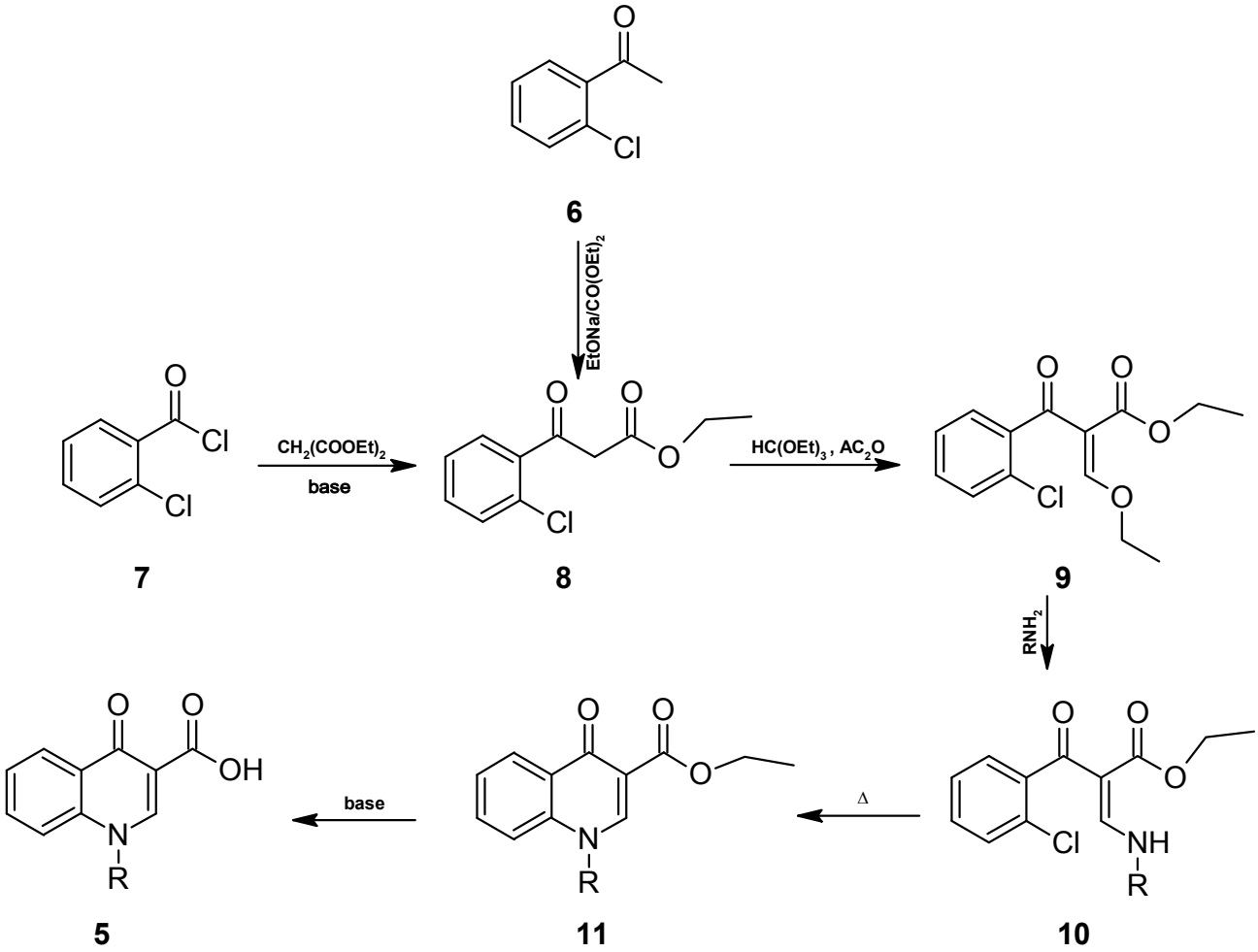
Generally, quinolones directly inhibit the DNA synthesis by binding to the enzyme-DNA complex. Quinolones also stabilize DNA strand breaks created by DNA gyrase and topoisomerase IV. Ternary complexes of drug, enzyme, and DNA block progress of the replication fork. The cytotoxicity of fluoroquinolones can be explained on the basis of a 2-step process: i) conversion of the ternary complex (topoisomerase quinolone-DNA) to an irreversible form, and ii)- production of a double-strand break by denaturation of the topoisomerase. The molecular basis necessary for the transition from step 1 to step 2 is still unclear (13).

Supercoiling and Topoisomerases

Supercoiling (14, 15) is the process in which chromosomal DNA is wound around itself making it possible for the 1-2 meter long chromosome to fit within the cell, and the supercoiled DNA has an effective diameter of about 1 μm.



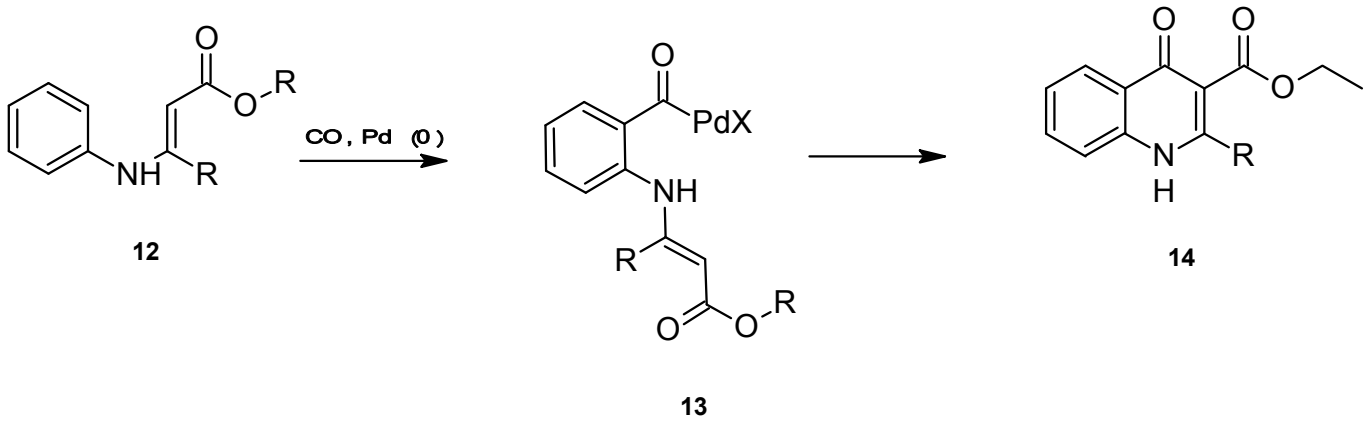
Scheme 1. Synthesis of 4-quinolones using the Gould-Jacobs reaction.



Scheme 2. Synthesis of 4-quinolones using Grohe-Heitzer reaction.

The winding and unwinding of DNA is accomplished by a class of enzymes called topoisomerases (topo). Supercoiling is achieved by the passage of one DNA strand through another; type I topoisomerase enzymes perform DNA passage after creating a single-strand break, while type II enzymes catalyse the passage of a double-stranded region of DNA through a double-stranded break in the helix. The supercoiling level in living bacteria is determined by the balancing activities of topo I and a topo II enzymes. The production of topo I and DNA gyrase is self-regulating which means that an overactivity of one encourages the transcription of the genes for the other enzyme. The strand passage is a critical feature to all topoisomerases as it requires the enzymes to cause breaks in the genetic material. These breaks are stabilized by covalent bonds between the 3′ end (eukaryotic topoisomerase I) or 5′ end of the newly formed break and the enzyme. Normally, these cleavage complexes are catalytic intermediates, present only in low concentrations and are therefore tolerated by the cell. However, conditions that significantly increase the physiological concentrations or lifetime of these breaks promote plenty of harmful side effects, including mutations, insertions, deletions and chromosomal abnormalities (16).

The DNA break is formed through a transesterification step which leads to the attachment of the 5′- phorphoryl group on DNA to the hydroxyl on Tyr-122 on GyrA (18). The DNA is cut in a sequence-specific manner, creating a 4-bp staggered break on opposite strands of the DNA. In a study by Morrison *et al.* (19) the cleavage of four double stranded DNAs was examined. Each double-stranded DNA strand was cleaved between T and G on the one strand; however, the cleavage on the complementary strand seemed to have no sequence specificity.



Scheme 3. Synthesis of 4-quinolone-3-carboxylic acid derivatives by palladium-catalyzed carbonylative heterocyclization.

Important structural features of antibacterial quinolones

It is really important to highlight the structural requirements for a quinoline pharmacophore to exhibit antibacterial activity, as presented in **Figure 1**.

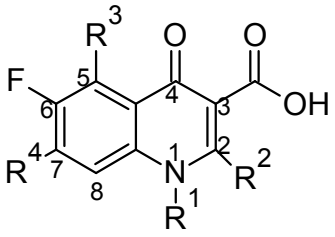


Figure 1. The most-commonly used structure of the quinolone molecule.

i. Position 1

This position is part of the enzyme-DNA binding complex, and has a hydrophobic interaction with the major groove of DNA (20). A cyclopropyl substituent is considered the most potent substitution here; the second important substitution for the required activity is the addition of a 2,4-difluorophenyl (21).

ii. Position 2

This location is very close to the site for the gyrase binding site (or topoisomerase IV) and it is well known that any added bulk inhibits the transport and results in a lower level of microbiological activity (20). Only a sulfur, incorporated into a small ring, has been able to replace hydrogen at the R-2 position (20).

iii. Positions 3 and 4

These two positions on the quinolone nucleus are essential for gyrase binding and bacterial transport and no other useful substitutions have yet been reported. Therefore, the 3-carboxylate and 4-carbonyl groups are considered essential for antimicrobial activity (22).

iv. Position 5

Substituents at this position of the basic quinolone nucleus appear to have the capacity to control potency. Electron-donating groups such as an amino, hydroxyl, or methyl were found to increase the *in vitro* activity against gram-positive bacteria (23).

v. Position 6

The substitution with a fluorine atom had markedly improved antimicrobial activity compared to the original quinolone agents, and gave rise to the now widely used and clinically successful fluoroquinolone compounds. Ledoussal *et al*. (24) suggested that the 6-H is equivalent in activity to the 6-fluoro analogue even at the enzyme level.

vi. Position 7

Five- or six-membered nitrogen heterocycles are the most commonly applied moieties to this position. This position is considered to be one that directly interacts with DNA gyrase (25).

vii. Position 8

Of particular interest is the observation that specific changes in position 8 appear to dramatically alter the initial target in fluoroquinolones. For example, a simple hydrogen as in ciprofloxacin, or a fused ring (for example, ofloxacin and levofloxacin that have a benzoxazine bridge between C-8 and N-1) typically

leads to high activity against topoisomerase IV, with little clinically useful activity against DNA gyrase.

The current knowledge of structure-activity relationships has been gathered through the past development of a large number of compounds within the quinolone class and goes on to improve the antimicrobial activity and extend the useful life of such clinically important compounds.

Dual targets mechanism of action

Quinolones act in a similar fashion against the two prokaryotic type II enzymes. They essentially block the topoisomerase catalytic cycle when the protein is covalently linked to the cleaved DNA (cleavage complex). Stabilization of the enzyme-DNA complex has been confirmed to block the progression of the replicative machinery and to create DNA lesions that induce a bacterial SOS response (26, 27). Amino acid mutations in the Quinolone Resistance Determining Region (QRDR) greatly reduce the affinity of quinolones for the enzyme-DNA complex. The most relevant positions in *E. Coli* are Ser83 and Asp87 in GyrA, and the corresponding Ser79 and Asp83 in ParC (28, 29). The structural features of the interactions between quinolone-gyrase-DNA complex were identified by the sequencing of additional mutant gyrA and gyrB genes that produce the altered quinolone susceptibility (30). Experimental evidence suggests that quinolones, in the presence of proper metal ions, bind single stranded DNA and GyrA with moderate affinity (31, 32). However, they bind efficiently to the gyrase-DNA complex (33). At the DNA level, cleavage is not required to stimulate drug binding: the structural distortion of the double helix, bound to the protein and stabilized by selective contacts with protein residues, appears to be sufficient to provide a favourable interaction site for quinolones (34). Additionally, the presence of quinolones alters not only the structural features of GyrA (binding site) but also those of the overall A2B2-DNA complex (32, 35) inducing modifications in the kinetic rates of different catalytic steps.

Differences between DNA gyrase and eukaryotic topo II

In eukaryotes, DNA gyrase and topoisomerase IV are functionally replaced by two isoenzymes: topoisomerase IIa and IIb, a 170 and a 180 kDa protein, respectively (3). These proteins share a similar catalytic cycle, the main difference being in the fact that the active form of eukaryotic topoisomerase II (Topo 2) is constituted by a homodimer. However, as supported by the extended sequence homology, Topo 2 can be seen as the fusion of the GyrA-GyrB or ParC- ParE subunits.

Common features in the mechanism of action of antibacterial quinolones and antitumor drugs have suggested that both compounds have a similar mode of interaction with the type II DNA topoisomerase-DNA complexes. In particular, comparison between quinolones and antitumor drugs of the epipodophyllotoxin family (etoposide and teniposide) has been considered, since neither type of drug is a DNA intercalator (36). Archaebacteria are considered as being an intermediate phylogenetic position between eukaryotes and eubacteria (37). In these systems, comparable sensitivity to poisons of each type II topoisomerase was observed and the DNA cleavage patterns induced by ciprofloxacin and etoposide were found to be very similar (38, 39). These findings strongly supported a common mode of interaction with the DNA- topoisomerase II complexes for ciprofloxacin and etoposide.

Quinolone-based anticancer derivatives

Quinolone derivatives represent a large number of antiproliferative agents exhibiting cytotoxicity through DNA intercalation, causing interference in the replication process (40-42). Actinomycin D, doxorubicin, mitoxantrone and streptonigrin are quinoline analogs possessing antibacterial or anti-cancer activity through DNA intercalation. Most of these drugs are currently used in the treatment of human malignancies targeting topoisomerase (types II) enzymes (43-45).

An interesting point which was highlighted in many quinolones' previous reviews, is: how can small molecules discriminate efficiently between such similar enzymes? It was found that a minute change in the quinoline structure enabled the drug to act on another different target. High throughput screening revealed that there are two major types of modification that appear to drive the quinolone action from antibacterial to anticancer: i) disruption of the zwitterionic properties of the compounds by modifying either the C7 basic substituent or the C3 carboxyl group, or both, will affect the electron density distribution and protonation equilibria; ii) increase in the number of aromatic/condensed rings is mostly lead to increase the affinity of quinolones for double-stranded DNA, while antibacterial

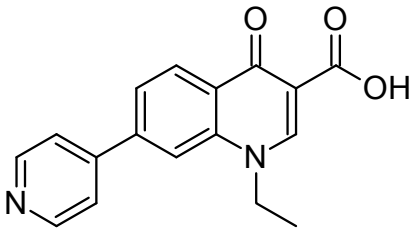
quinolones interact better with single-stranded DNA (46).

Historic development of cytotoxic quinolones

Topoisomerase II is the primary target for several classes of antineoplastic drugs (47, 48, 49). These agents are widely used for the treatment of human cancers (47, 48, 49) and their clinical efficacies correlate with their abilities to stabilize covalent enzyme cleaved DNA complexes that are intermediates in the catalytic cycle of the enzyme (47-51). While quinolone-based drugs have been developed extensively as antimicrobial agents (targeted to DNA gyrase, the prokaryotic counterpart of topoisomerase II) (52, 53), these studies provided evidence that quinolones may have potential as antineoplastic drugs. When comparing the known sequences of topoisomerases from bacteria to mammals, the sequences appear to be similar around active site tyrosine, not only among type II topoisomerase but also among type I. They also share the same mechanism of cell killing which is performed by trapping topoisomerase II in an intermediary cleavable complex with DNA, termed the "cleavable complex," which is detected as DNA double strand breaks (54). The pioneering study in this field showed that Ciprofloxacin and Ofloxacin interfere only slightly with the function of Calf Thymus Topo II, the prokaryotic Gyrase being approximately 100-fold more sensitive to inhibition than its eukaryotic counterpart (55).

Rosoxacin, a 7-(4-pyridinyl)quinolone derivative, showed great interest in treating gonococcal infections (56). Moreover, the 7-(4-pyridinyl) derivatives produced an unacceptable toxicity profile *in vitro* inducing a genetic toxicity endpoint (57).

Since that time, different directions were followed to elucidate the structural activity relationships responsible for the interference of quinolones with eukaryotic topoisomerase II.



Rosoxacin

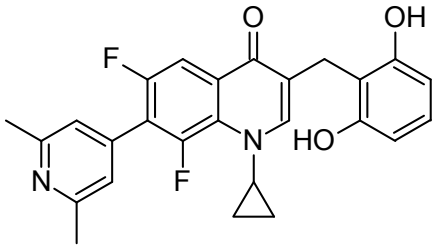
Figure 2. Structure of the cytotoxic quinolone Rosoxacin.

Important structural features of anticancer quinolones

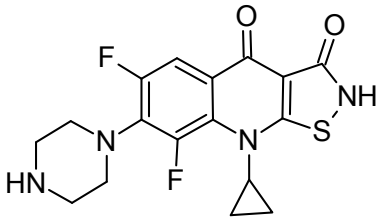
i. Position 3

It is well established that, in antibacterial quinolones, the 3-COOH or its isosteric replacement is a fundamental requirement for activity. However, this functional group can be replaced even with hydrogen and effective poisoning of Topo 2 can still be maintained. It seems that, to inhibit the eukaryotic enzyme, the basic requirement is the coplanarity of the C-3 substituent with the quinoline ring. Thus, although in 3-H derivatives small substituents can be beneficial at C-2, no such residue can be introduced if the carboxyl group is present, as it will destabilize the coplanar orientation of the acidic moiety (58). Removal of the carboxylic acid group opened up new synthetic opportunities. In fact, introduction of a phenyl group or a related heteroaromatic ring at C-2 enhanced Topo 2 poisoning activity (59). The distance between the two aromatic moieties is apparently crucial, as only a methylene linker allowed the maintenance of biological activity. Further increase in cytotoxic activity was obtained upon introduction of hydroxyl substituents into the C-2 phenyl group. In particular, the 2,6-dihydroxy benzyl derivative (Win 64593) is the most active quinolone derivative. In quinolones carrying the carboxyl group, the acidic moiety can participate, with the ketone at position 4, in chelating metal ions or can be involved in hydrogen binding with the target macromolecules.

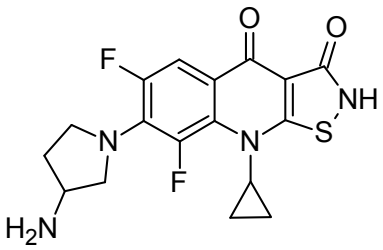
The substitution of nitrogen at position 1 with a sulfur atom leads to inactive derivatives (58). However, as reported, other positions can support the introduction of this heteroatom without the loss of activity. An example is represented by the isothiazolo quinolones A-65281 and A-65282 (60). These derivatives exhibit a heterocyclic moiety added to the quinolone system, which can be considered as a modification of the carboxyl group. It is worth noting that, in spite of the fact that these derivatives do not exhibit an aromatic substituent at position C-7, they are active against both bacterial and eukaryotic type II topoisomerases.



Win-64593



A-65281

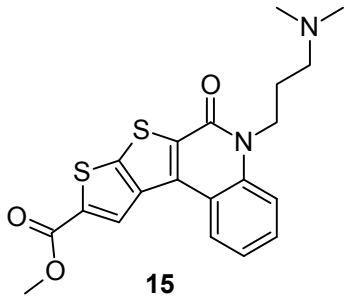


A-65282

More recently, (61) derivatives bearing a 3-dimethylaminopropyl substituent on the quinolone nitrogen and a methoxycarbonyl group at position 9 (compound **15)** exhibited prominent antitumor activity. Cytotoxicity was reduced when an anilido substituent was present at position 9 (compound **16)**.

ii. Positions 6 and 8

A study done in 1991 (62) showed that 6,8-difluoroquinolones were potent in affecting the eukaryotic topoisomerase II. Followed by another study (63) showing the contribution of the C-8 fluorine to drug potency, the authors compared the effects of CP-115,955 [6-fluoro-7-(4-hydroxyphenyl)-1-cyclopropyl-4-quinolone-3-carboxylic acid] on the enzymatic activities of *Drosophila melanogaster* topoisomerase II with those of CP-115,953 (the 6,8-difluoro parent compound of CP-115,955). Results showed that removal of the C-8 fluoro group decreased the ability of the quinolone to enhance the enzyme-mediated DNA cleavage ~2.5-fold.



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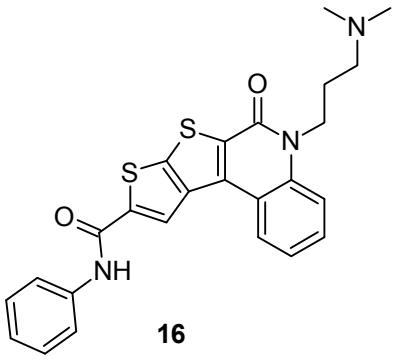

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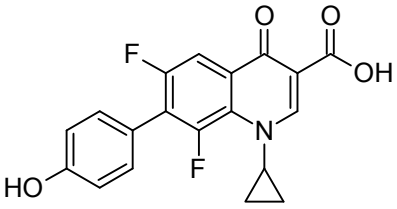
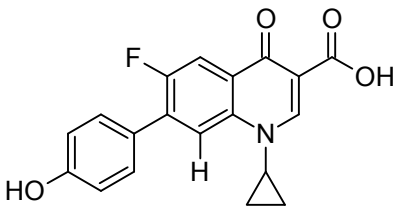


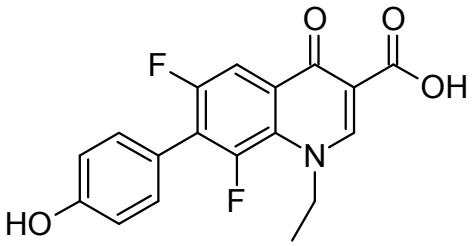
CP-115,953



CP-115,955

iii. Ring position N-1

Considerable evidence (64) indicates that the presence of a cyclopropyl as opposed to an ethyl group at position N-1 increases quinolone potency against DNA gyrase and by comparing the DNA cleavage-enhancing activity of ciprofloxacin with that of norfloxacin or the activity of CP-115,953 with that of CP-67,804, it was determined that the substitution of an ethyl group at N-1 decreased quinolone potency against calf thymus topoisomerase II between -30- to -40- fold, respectively.



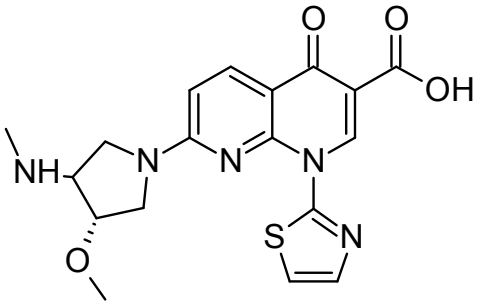
CP-67,804

i. Substitution at C-7

A variety of substitutions were also introduced at position C-7. In fact, the presence of a basic amino group in the aliphatic cyclic substituent at position C-7 is a strict requirement for antibacterial activity. Additionally, modifications at this position were shown to play a key role in directing the drug preferentially towards Gyr or Topo 4 (65). These results suggested a direct interaction of this portion of the quinolone with the enzyme. Conceivably, the C-7 substituent should affect the prokaryotic/eukaryotic enzyme selectively. Indeed, methyl substituents at the C-7 piperazine group influenced potency against the mammalian enzyme. In fact 3,5-dimethylpiperazinyl derivatives were active in stimulating enzyme-mediated DNA cleavage only in the *trans* configuration. This was particularly interesting because *cis*- or *trans*-methyl substitution on the piperazine had little effect on the activity against Gyr, suggesting that only in the mammalian enzyme an asymmetric barrier exists which influences productive quinolone interaction and favors the less bulky *trans*-3,5-dimethylpiperazine substituent at C-7 (66). Parallel studies demonstrated that compounds with C-7 pyrrolidine substituents were more cytotoxic than those with piperazine substituents (67). This is actually in line with observations made on derivatives related to the earlier cytotoxic quinolones (68). Looking both at the stimulation of DNA cleavage and the cytotoxicity against cultured mammalian cells, it was observed that the presence of an aromatic group contributed greatly to drug activity. In particular, 4'-hydroxyphenyl substituent at the C-7 position was found to be critical for potency towards the mammalian Topo 2 (64). From this series, **CP-115953**, 6,8-difluoro-7-(4'-hydroxyphenyl)-1-cyclopropyl-4-quinolone-3-carboxylic acid, was found to be the most cytotoxic quinolone derivative (62).

Quinolone derivatives as topoisomerase inhibitors

Vosaroxin (69) (formerly **voreloxin**) is an anticancer quinolone derivative that intercalates DNA and inhibits topoisomerase II, inducing site-selective double-strand breaks (DSB), G2 arrest and apoptosis. The quinolone-based scaffold differentiates vosaroxin from the anthracyclines and anthracenediones. It has been reported that vosaroxin induces a cell cycle specific pattern of DNA damage and repair that is distinct from anthracycline and doxorubicin (69).



Voreloxin

In 1989, Kyorin/Kyowa-Hakko described thiazoloquinolone carboxylic acids with an impressive anticancer profile (compound A) (70). The clinical candidate had exhibited favorable drug-like properties in different animal models in preclinical studies.

Compound A, like other quinolone carboxylic acid derivatives, was presumed to interact with topoisomerase II via its β-keto acid functional group by chelating with the Mg^{2+} ion to inhibit the enzyme. Considering this mode of action, (71) we hypothesized that a quinolone with a β-diketo functionality may be able to mimic the action of β-ketoester functionality, thus potentially providing

the same level of complexation with the Mg^{2+} ion at the active site of the topoisomerase II. Also, due to the absence of free carboxylic acid, the target quinolone may cause less gastric damage when used via oral administration.

The design of target compounds was based on a structure-based design approach using the Hyperchem-3™ molecular modeling program. By applying Molecular Mechanics Optimization (MMO) and Molecular Dynamic Option methods we were able to identify linear tricyclic quinolones with β-diketo components that matched the angular feature of Compound A. In this respect, compound B, 9-benzyl-7-fluoro-3-hydroxythieno[4,5-*b*]quinoline-4(9*H*)-one, at its optimized steric/energetic configuration, was found to have the best match with a perfect 3-point overlay with compound A as depicted in **Figure 3**.

Compound B and its derivatives were synthesized in our group using the Gould Jacob method. This series of compounds displayed promising cytotoxic activity against several cancer cell lines (72).

Compound C also was designed to act in the same manner as compounds B and A applying the most important structural features which is the coplanarity of the β-diketo functionality.

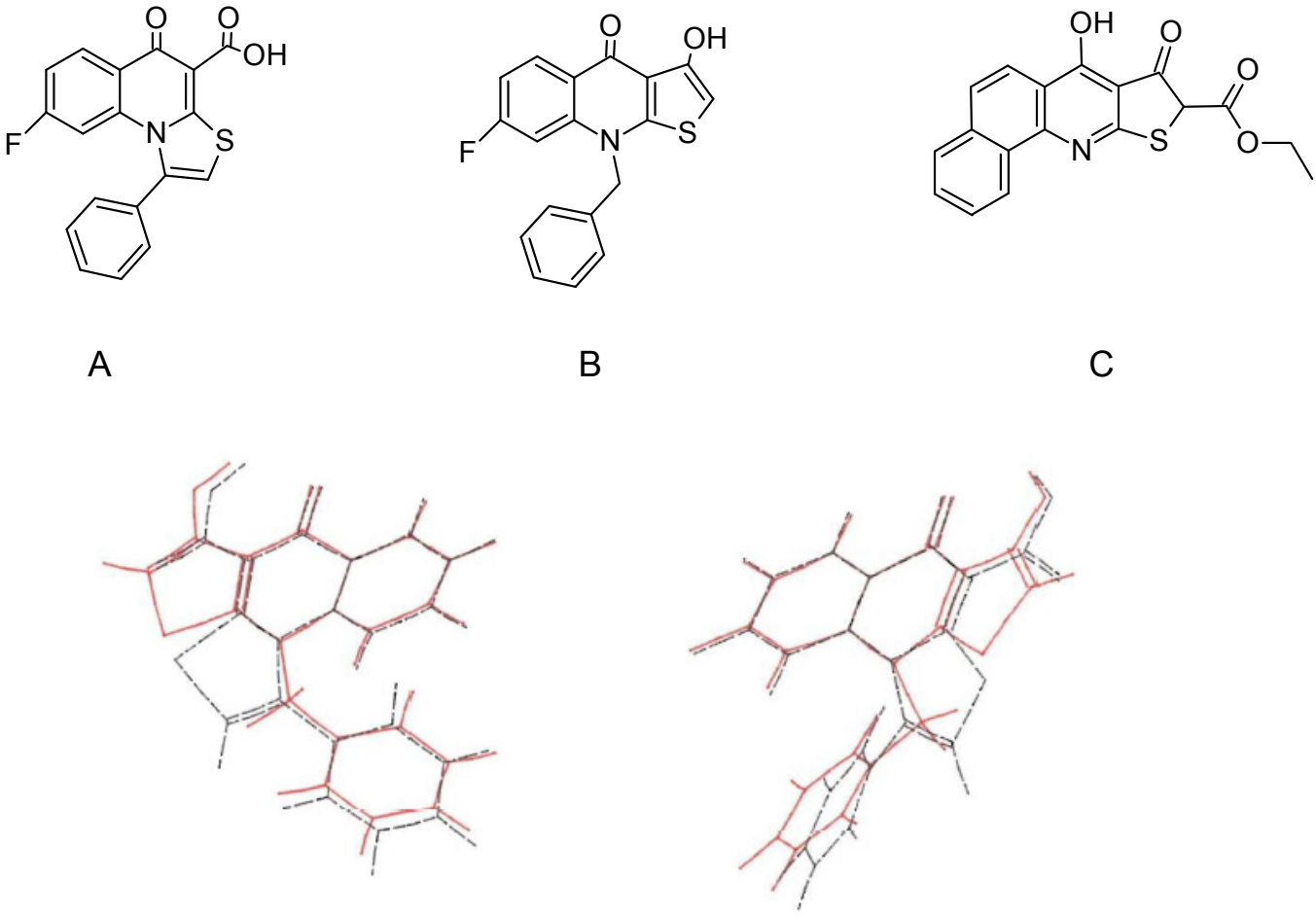


Figure 3. Different overlays of the structure of Compound A (dotted line, black) and Compound B (solid line, red), and structure of Compound C (designed derivative).

We were interested in running the molecular docking on compound A which showed interaction with the ATP binding site of the protein with docking energy equal to -7.39 kcal/mol. Also, compound A was able to donate non-bonding electrons to Mg^{2+} (73). Some of our synthesized structures (73) gave better interaction with the ATP binding site with reasonable affinity, with C possessing the lowest docking energy.

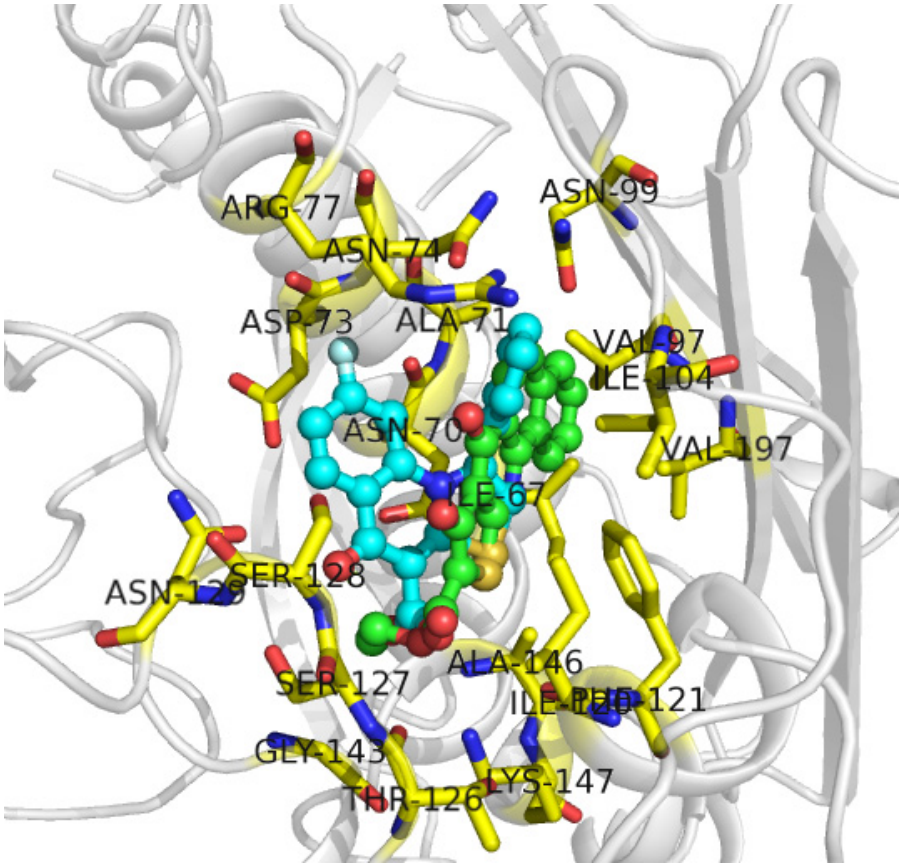


Figure 4. Overlapping image of Compound C (green color) and Kyorin/Kyowa Hakko compound (A-blue color) at the ATP binding site of 1QZR.

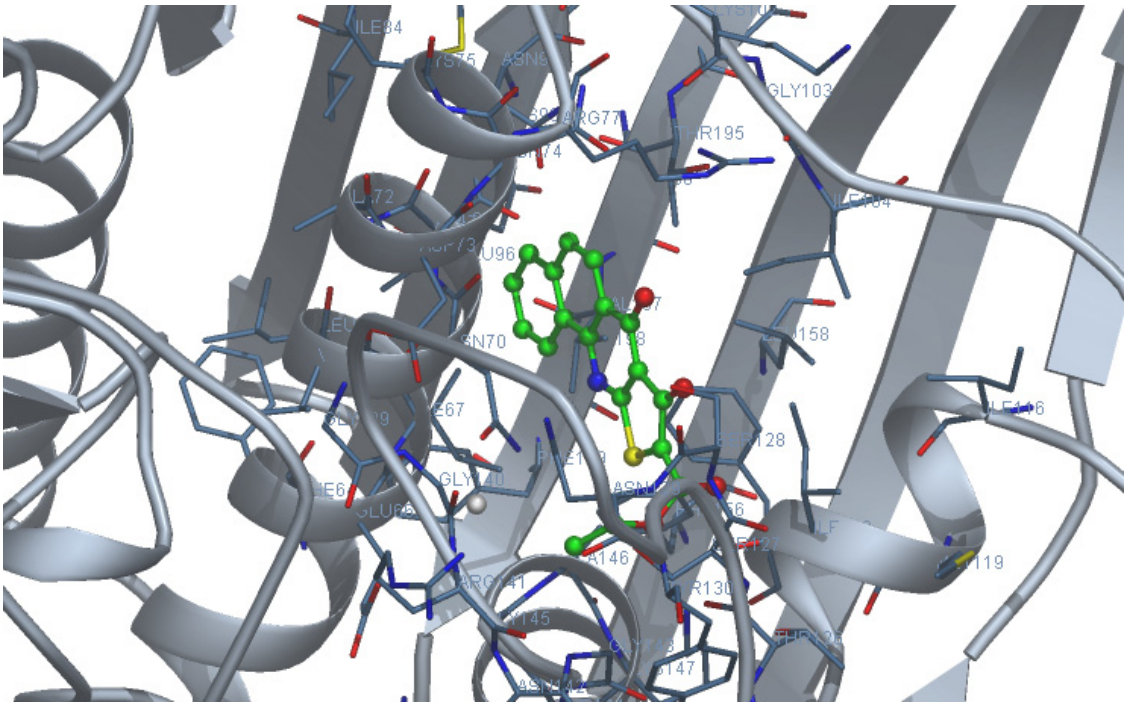
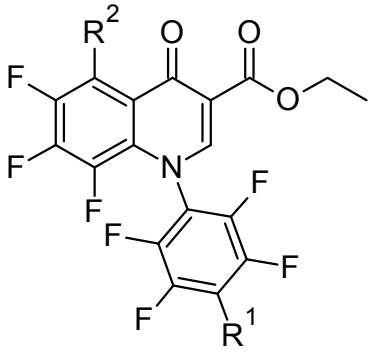


Figure 5. Compound C docked at the ATP binding site of 1QZR.

These derivatives did not show cytotoxicity against cancer cell lines. We attributed this finding to a solubility problem and efflux pump mechanisms.

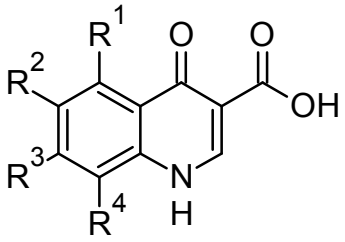
Signal transducers and activators of transcription (STATs) inhibition

Signal transducers and activators of transcription (STATs) are an important family of molecules that mediate signal transduction in cells (74, 75). Accumulating evidence indicates that STAT family members play important roles in carcinogenesis and, in particular, STAT3 has emerged as a good target for cancer therapy (76-78). Very recently, a high throughput screening approach allowed the identification of the highly fluorinated quinolone derivative **17** (R_1= R_2 = F) that inhibits the STAT3 pathway and causes cell apoptosis (EC_{50} =4.6 μM). Lead optimization with modification of the phenyl moiety led to the identification of the 4-cyanophenyl derivative **18** (R_1=CN, R_2=H) with a 30-fold increase in potency (EC_{50} = 170 nM) in which cell apoptosis induction correlates well with inhibition of steady state and cytokine induced JAK and STAT3 activation (79).



17,18

In a receptor-based virtual screening of around 70.000 compounds, some 3-carboxy-4(1*H*)-quinolones were revealed to be human protein kinase CK2 inhibitors (80). Protein kinase CK2 participates not only in the development of some types of cancers but also in viral infections and inflammatory failures. Thus, quinolones could be considered not only potential antitumor, but also anti-infectious and anti-inflammatory drugs. The compounds with high docking scores were selected for *in vitro* tests, and compounds **19** and **20** displayed IC_{50} values of 0.3 and 1 μM, respectively. These findings led to investigating other derivatives of this class in order to determine the effects of certain substituents on their inhibitory activity.



19 : R^1 = R^2= R^4 = Cl, R^3 = H

20 : R^1 = R^2 = H, R^3-R^4 = -CH=CH=CH=CH-

Quinolone scaffold–based antiviral agents

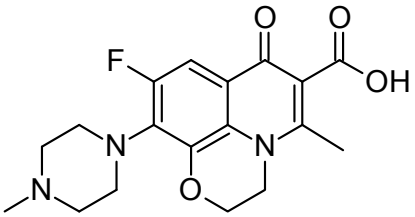
Quinolones are not new in the antiviral field. Their previously reported data on binding with bacterial chromosome had strengthened the hypothesis that these drugs could also bind to the viral nucleic acid, and prompted the investigation of their antiviral activity (81-86).

Many structures containing the basic quinolone carboxylic acid template and different lipophilic substituents were patented as antiviral agents: most of them were tested against human immunodeficiency virus-1 (HIV-1) and were claimed to be effective in the treatment or prophylaxis of Acquired Immune Deficiency Syndrome (AIDS) (87-89). However, their mechanism of action was not reported.

Quinolone-based anti HIV agents

An earlier work done by Furusawa *et al.* (90), reported that several fluoroquinolones protect cells from HIV-mediated cytotoxicity. The results showed that after ofloxacin [**21]** treatment the protected cells were able to survive for extra 3 months without any loss in cell viability. The L-isomer of ofloxacin (DR-3355) (91) was also reported to protect cells from HIV-1 mediated cytolysis in which the surviving cells were unable to produce an infective virus and also lost expression of the CD4 antigen (90).

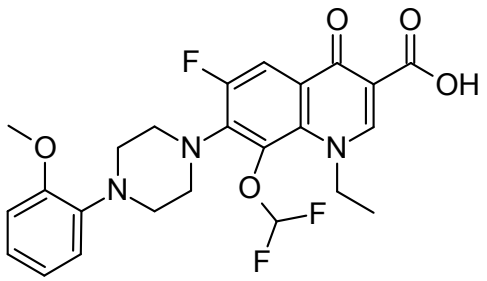
The exact mechanism of action of antiviral quinolones is still unknown. The following classification is done according to the authors' postulations.



21

Integration of proviral DNA into mRNA

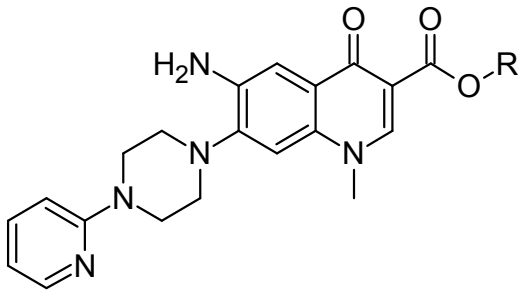
Baba *et al*. (92) found that antibacterial fluoroquinolones can exhibit antiviral activity as well. Analogues, bearing 8-difluoromethoxy-1-ethyl-6-fluoro-1,4-dihydro-7-[4-(2-methoxyphenyl)-1-piperazinyl]-4-oxoquinoline-3-carboxylic acid (**22**), exhibited EC_{50} < 50 nM in chronically infected cells. Compound **22** suppressed tumor necrosis factor alpha (TNF-a)-induced HIV-1 expression in latently infected cells (OM-10.1) and constitutive viral production in chronically infected cells (MOLT-4/III_B) at a concentration of 0.8 mM. It was reported that compound **22** could also inhibit HIV-1 antigen expression in OM-10.1 and MOLT-4/III_B cells at the same concentration (92). Inhibitors of this step, which is considered a crucial step in HIV replication, will be able to suppress HIV replication not only in acutely but also in chronically infected cells (93).



22

Interference with a postintegrational target of the HIV-1 replication cycle

Much of the literature (94-96) has addressed the potential of 6-aminoquinoline series to inhibit the HIV replication cycle. The prototype was compound **23.** The synthesized derivatives showed very strong activity on HIV-1 acutely infected MT-4, CEM, and PBMCs cells as well as on chronically infected HuT78. A potent antiviral activity was also observed in latently HIV- 1 infected M/M cells at drug concentrations as low as 40 ηg/mL. This activity was further confirmed in an *in vivo* model for HIV-1 latency, which provided encouraging evidence for the use of quinolones in the control of HIV-1 infection (97). The studies on the mechanism of action revealed that 6-DFQs interfere with a postintegrational target of the HIV-1 replication cycle.

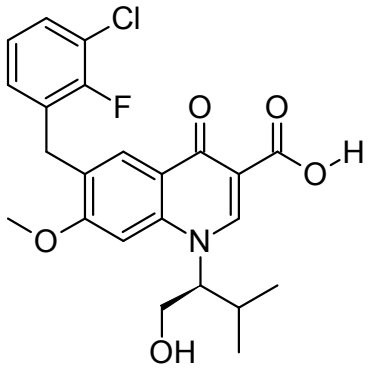


23

HIV-1 integrase inhibitors

HIV-1 integrase (IN) (98-103), along with HIV-1 reverse transcriptase and HIV-1 protease, are essential enzymes for retroviral replication and represent important targets for interrupting the viral replication cycle.

IN is an attractive target because it has no counterpart in mammalian cells; therefore, most IN inhibitors should possess high selectivity and low toxicity. Quinolone derivatives, as a class of HIV-1 inhibitors, have become of great interest due to their high molecular versatility, easy synthesis at low cost and on a large scale, and well-recognized biochemical properties that make them very suitable pharmacophore structures. The first quinolone-based structure Elvitegravir (**GS-9137**) **w**ith very strong antiretroviral properties owes its anti-HIV activity exclusively to the inhibition of the viral enzyme integrase.



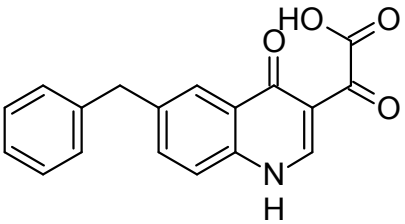
GS-9137

The quinolone-based structure was also assumed to be a new pharmacophore for designing new generation HIV-1 IN inhibitors. Generally, a diketo acid moiety (*γ*-ketone, enolizable α-ketone, and carboxylic acid) is considered to be essential for the activity of integrase inhibitors (101) and the structures of diketotriazole (104) diketotetrazole (105) diketopyridine (106) and 7-oxo-8-hydroxy-(1,6)-naphthyridine (107, 108) were reported to be bioisosters of the diketo acid pharmacophore. It was found that the carboxylic acid could be replaced not only with acidic bioisosters, such as tetrazole and 1,2,3-triazole, but also by a basic heterocycle bearing a lone pair donor atom, such as a pyridine ring. Also, The enolizable ketone at the α-position of diketo acids can be replaced with a phenolic hydroxyl group, showing that the α-enol form of each diketo acid is its biologically active coplanar conformation. The 4-quinolone-3-glyoxylic acid **24** was treated as a new scaffold that maintained the coplanarity of diketo acid functional groups. Interestingly, not only the 4-quinolone-3-glyoxlic acid but also its precursor 4-quinolone-3-carboxylic acid **25** showed integrase inhibitory activity (101). The 4-quinolone-3-carboxylic acid only had two functional groups, a *β*-ketone and a carboxylic acid, which were coplanar. This result showed that the coplanar monoketo acid motif in 4-quinolone-3-carboxylic acid could be an alternative to the diketo acid motif, and this in turn provided a novel insight into the structural requirements and the binding mode of this type of inhibitor (108).

The emergence of resistant mutants against available antiretroviral drugs brings out the need to develop new therapeutic targets. HIV integrase inhibitors open up a new way as a promising class of antiretroviral drugs. They act by inhibiting the action of the integrase, a viral enzyme that inserts the viral genome into the DNA of the host cell preventing formation of the provirus. The replicative life cycle of the HIV virus requires the integration of viral DNA into genomic DNA of the host cell, a process which is mediated by the viral protein integrase (IN). The development of inhibitors of IN activity constitutes a new challenge for the treatment of HIV-1 infection. Diketoacid functionality is critical for enzyme inhibition. Their derivatives as well constitute one of the most successful classes of IN inhibitors that exhibit potent strand transfer inhibition as well as good antiviral activity. Recently (109), a series of potential HIV integrase inhibitors derived from quinolone antibiotics was described. The structurally optimized and highly potent monoketo compound **GS-9137** was identified as the most promising candidate, exhibiting potent inhibitory activity against integrase-catalyzed DNA strand transfer. **GS-9137** was shown to be well tolerated in healthy volunteers and HIV-infected patients. Moreover, monotherapy resulted in substantial antiviral activity in infected subjects. The agent is undergoing phase III clinical evaluation for the treatment of HIV-1 infection.

Another IN inhibitor (110) **GS-9160** has potent and selective antiviral activity in primary human T-lymphocytes producing an EC_{50} of ~2 nM with a selectivity index (CC_{50}/EC_{50}) of ~2000, but later on **GS-9160** suffered from unfavorable pharmacokinetic properties.

Diketoacid compounds (DKAs) constitute a promising class of HIV IN inhibitors with *in vivo* anti-viral activity (111, 112). Styrylquinolines (SQs) are another group that are also capable of IN inhibition (113, 114). Several compounds of both classes are under clinical trials including **S-1360** (115) or **L-870810** (DKA) (116), and **FZ-41** (SQ) which is under earlier stages of drug development (117). Comparison of these two classes shows some similarities, but they also differ in their inhibitory activity. Compound **SQ1**, which is synthesized in an attempt to mimic the DKA pharmacophore in the styrylquinoline series, showed to be inactive *in vitro* against IN (118). On the other hand, this compound exhibited a significant antiviral activity in the *in vivo* experiment (118).



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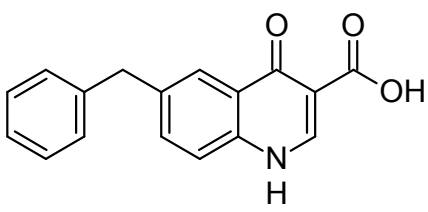

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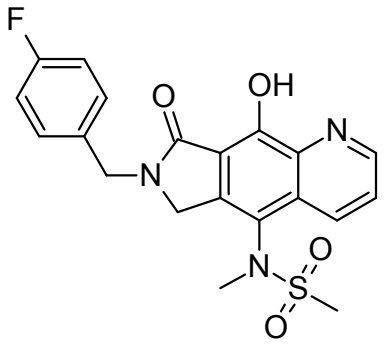
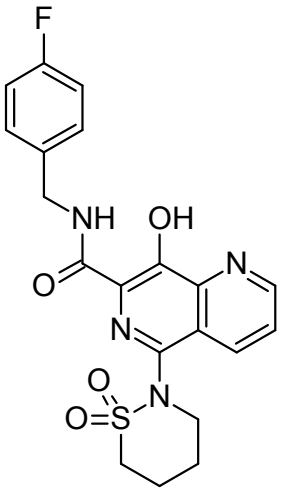
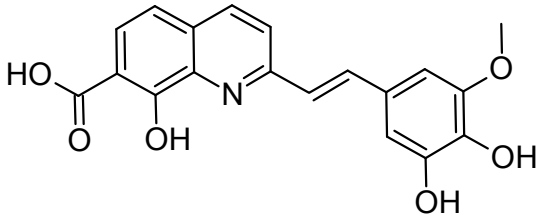


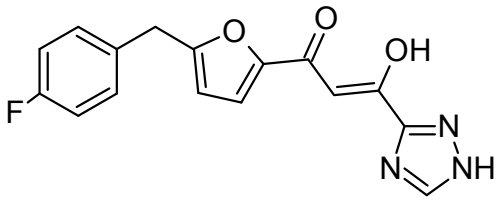
GS-9160



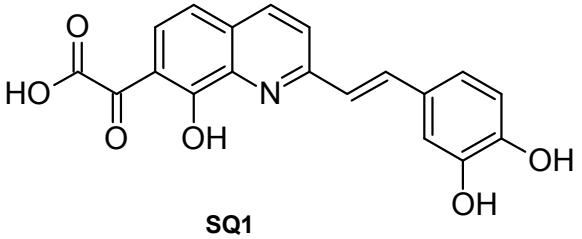
L - 870810



FZ-41



S -1360



SQ1

Moreover, Engels *et al.* (119) described the microwave-assisted synthesis of fluoroquinolone nucleosides **26, 27** and their evaluation as HIV-1 integrase inhibitors. These compounds were proved to be inactive against HIV-1 replication at subtoxic concentrations in the MT-4/MTT assay.

In order to draw a clear SAR among the compounds possessing diketoacid group that act as HIV integrase inhibitors, Pasquini *et al.* (120) explored this field by designing and synthesizing novel quinolones with the aim to obtain more potent compounds and to get more information concerning SAR within this class of HIV-1 integrase inhibitors (120). Results showed variation of the substituent at the N-1 has little influence on the IN inhibitory activity, while it affects their antiretroviral potency.

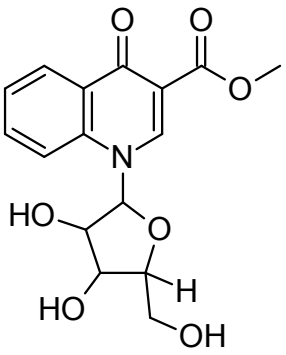
This effect of the N-1 substituent on inhibition of the IN catalytic activity indicates that this part of the molecule may not be engaged in relevant interactions with the target enzyme. Conversely, favorable physiochemical and pharmacokinetic properties, responsible for marked improvements in antiviral activity of these compounds, depend on the substitution pattern at N-1, with the (*1S*)-1-hydroxy-3-methyl-2-butyl side chain conferring the optimal profile. It has previously been reported that the integrase inhibitor **S-1360** selectively inhibits the ST step via a mechanism similar to that of the DKA (121). Accordingly, the activity of quinolones is likely due to sequestration of divalent metal ions within the IN active site in complex with viral DNA (121). Although the crystallographic structure of IN in complex with viral DNA has not yet been resolved, it has been reported that Tn5 transposase (Tnp), which belongs to the superfamily of polynucleotidyl transferases, as does IN, can be considered as an excellent surrogate model for studying the mechanism of action of ST inhibitors (121). In this context, docking calculations of many synthesized derivatives together with Elvitagravir were performed on the Tn5 Tnp-DNA complex by following the computational protocol described by Barreca *et al*. (121). In its best docking pose, the carboxylate group in Elvitagravir hydrogen-bonded to H329, displayed chelation with the metal ion that is located between E326 and D97, and the *β*-ketone oxygen along with the fluorine atom coordinate with the other metal ion. It was found that the binding mode proposed for Elvitagravir was in accordance with its reported mutation (120).

Quinolones as anti-HCV agents

Antiviral drugs can be classified into direct and indirect agents; the direct agents target the structural components or enzymes encoded by the virus while the indirect antiviral agents target the host cell components (immunomodulators etc.) (122). In the therapy of HCV infection, inhibitors of the NS3 protease, the NS5B polymerase, and the viral RNA are the most intensively explored. The actual treatment of HCV will be a combination of drugs of variable mechanisms in order to reduce the emergence of resistance (122). The latest developments in the discovery of quinoline-based agents to treat HCV are reviewed, with special focus on direct small-molecule antiviral drugs, from a medicinal chemistry perspective.

As is the case with HIV, efforts to develop anti-HCV agents have focused on the inhibition of key viral enzymes. In fact, and because of the proven success of protease inhibitors in the treatment of HIV, a large number of pharmaceutical companies have focused on the HCV NS3 proteolytic enzyme. HCV possesses two proteolytic enzymes: NS2/3 and NS3/NS4A. However, NS2/3 has received little attention as an antiviral target because of the incomplete knowledge of the NS2/3 cleavage process. Furthermore, NS2/3 processing is partially mediated by host cell proteases, making it a less attractive target for drug development (123, 124).

In addition to the HCV protease, NS3 possesses an RNA helicase domain. HCV helicase represents a relatively new and still unproven antiviral target. While, several studies on the *in vitro* activity of helicase inhibitors have been reported, there is no report on clinical effectiveness of these molecules(122).



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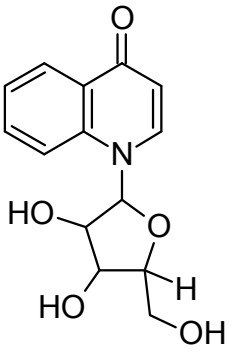
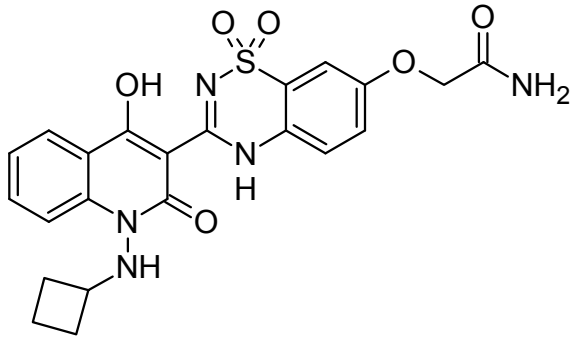


27

HCV NS5B polymerase inhibitors

The viral RNA dependent RNA polymerase (RdRp) encoded by the non-structural protein 5b (NS5B) is essential for viral replication. Several specific NS5B polymerase inhibitors (125) have shown promising results in clinical trials and the polymerase is considered a well validated drug target. Reported inhibitors are either nucleoside analogs which target the active site or non-nucleoside inhibitors (NNIs) that bind to one of the four allosteric sites (126, 127).



A-782759

A-782759, a 2-(1-aminoquinolone-3-yl) benzothiadiazine derivative, was identified as an inhibitor of HCV NS5B RdRp (128). The combination of Hepatitis C virus (HCV) polymerase **A-782759** with either Boehringer Ingelheim HCV NS3 protease inhibitor **BILN-2061** or interferon (**IFN**) (129, 130) have shown interesting additive to synergistic relationships over a range of concentrations of each of the two combined drugs. Treatment of HCV replicon with **A-782759**, **IFN** or **BILN-2061** for about 16 days resulted in dramatic reductions in HCV RNA (5.1, 3.0 and 3.9 log RNA copies, respectively). Surprisingly, none of these compounds when tested alone showed any replicon RNA reduction. Results showed that a monotherapy with either drug alone possibly results in development of resistant mutants. However, a combination therapy lowers the likelihood of resistance development (131).

Antiviral quinolones are promising compounds in the search for new therapeutically effective agents. Diketo functionality seems to play an important role in targeting the viral enzymes. The information gained so far will be useful for future rational drug design aimed at developing new compounds with optimized antiviral activity.

CONCLUSION

The 4-quinolone scaffold exhibits many pharmacological profiles. Beside the antibacterial activity, there are reports on antiischemic, anixolytic, antitumor, and antiviral activities of this class of compounds. In this review we have tried to highlight the most important pharmacological properties (antibacterial, antitumor and antiviral activities) of quinolones. SAR studies revealed that very fine changes in the main skeleton, as well as changing the fused rings will substantially affect the pharmacological activity profiles of these compounds.

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