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A REVIEW ARTICLE ON RECENT CHANGES IN FLUOROQUINOLONES

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Abstract:

Quinolones are potent antimicrobial agents with a basic chemical structure of bicyclic ring. Fluorine atom at position C-6 and various substituents on basic quinolone structure, namely norfloxacin, ciprofloxacin, levofloxacin and other agents and target molecules of quinolones and fluoroquinolones are bacterial gyrase and topoisomerase IV enzymes. Fluoroquinolones introduced in clinical trials namely avarofloxacin, delafloxacin, Finafloxacin, zabofloxacin and nonfluorinated nemonoxacin.

Fluoroquinolones endure of the most important kind of antibacterial agents and the emergence of more virulent and resistant strains of bacteria by development of mutated DNA, binding proteins, efflux pump mechanism. The new generations of fluoroquinolones with a spectrum activity includes Gram +Ve Gram -Ve and atypical bacteria. Many fluoroquinolones have other biological effects such as anti-HIV, anti-fungal, anti-plasmodic and anti-tumor activity.

Keywords: *Chemical structure, Clinical trials, Pharmacokinetics, Quinolones, Safety, Tolerability, Toxicity*

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INTRODUCTION:

Quinolones are useful synthetic antimicrobials first developed in the 1960s. As several agents have been synthetised by modification of normal bicyclic chemical structure. Quinolones and fluoroquinolones are classified based on their chemical structure, antibacterial spectrum and pharmacokinetic features. Each agent inhibits bacterial DNA synthesis by forming a pyramidal or triangular complex with a DNA molecule and gyrase and topoisomerase IV enzymes, thus blocking bacterial DNA supercoiling [1, 2, 3].

Based on the development of new derivatives on the antimalarial quinines by George Lesher leads to discovering nalidixic acid in 1962 as antibacterial agent. [4] Nalidixic acid, the prototype of this series, was first clinically useful drug in this series as urinary tract antibacterial agent. This is characterized by being with higher effectiveness against only Gram-negative bacteria, with poor serum and tissue concentrations after oral administration and limited spectrum against Enterobacteriaceae. [4, 5]A revolution in the spectrum of quinolones happened after the discoveryof 6-fluoro analogues that created the broad spectrum fluoroquinolones. [6]

Fluoroquinolones is regarded as one of the most common drugs used nowadays in treatment of various bacterial infections including urinary tract, upper and lower respiratory tract, skin, bone, soft tissue infections as well as community acquired pneumonia. [7] Apart from their typical antibacterial activity, fluoroquinolones also mentioned diverse atypical biological profiles as anti-tumor, [8, 9]anti-tubercular, [10, 11] anti-HIV, [12] anti-malarial, [13]and anti-Alzheimer activities. [14]

In spite of the fact that numerous fluoroquinolone agents have been produced in the last decades, only a few of them are marketed, and some of them have been withdrawn or restricted because of their toxicity [15]. The most frequent reasons for withdrawal included tendinitis after treatment with pefloxacin; rashes appeared after Sparfloxacin and clinafloxacin therapy; electrocardiogram disorders such as QTc prolongation occured during grepafloxacin administration; gatifloxacain and clinafloxacin therapy led to dysglycemia; hemolysis occured during temafloxacin administration; hepatotoxicity was found in trovafloxacin treatment [2, 15, 16].

Table 1 Pharmacokinetic features of quinolones

Quinolone Agents	Protein binding(%)	Urinary Fraction(%)	Bioavailability(%)	Cmax (mg/L)	t1/2(h)	Ref.
Ciprofloxacin	20-40	40-50	70	4.3	4	[17]
Levofloxacin	24-38	87	99	6.2	6-7	[17]
Sparfloxacin	45	10	92	1.1	20	[17]
Trovafloxacin	76	6	88	2.1	9.6	[17]
Moxifloxacin	50	20	90	4.5	12	[17]
Gatifloxacin	20	72	96	3.8	7.8	[17]
Avarofloxacin	65	12	65	2	14	[18]
Delafloxacin	16	n.a.	n.a.	10	12	[19]
Finafloxacin	n.a.	33	n.a.	11	10	[20]
Zabofloxacin	n.a.	n.a.	n.a.	2	8	[21]

Mechanism of Action

Fluoroquinolones inhibit the replication and transcription of bacterial DNA, which finally led to eliminate in cell death. [22] They inhibit the activity of DNA gyrase, an essential adenosine triphosphate-hydrolyzing topoisomerase II enzyme and also prevent the detachment of gyrase from DNA. [23] The topoisomerase inhibitors exert their bactericidal activity by interacting with the DNA. [24, 25]

During the processes of replication and transcription, enzymes called helicases uncoil the DNA double helix leading to additional supercoiling of the remaining DNA double helix. A tension is created in this remaining double helix, which must be recognize in order to continue the process. [25]

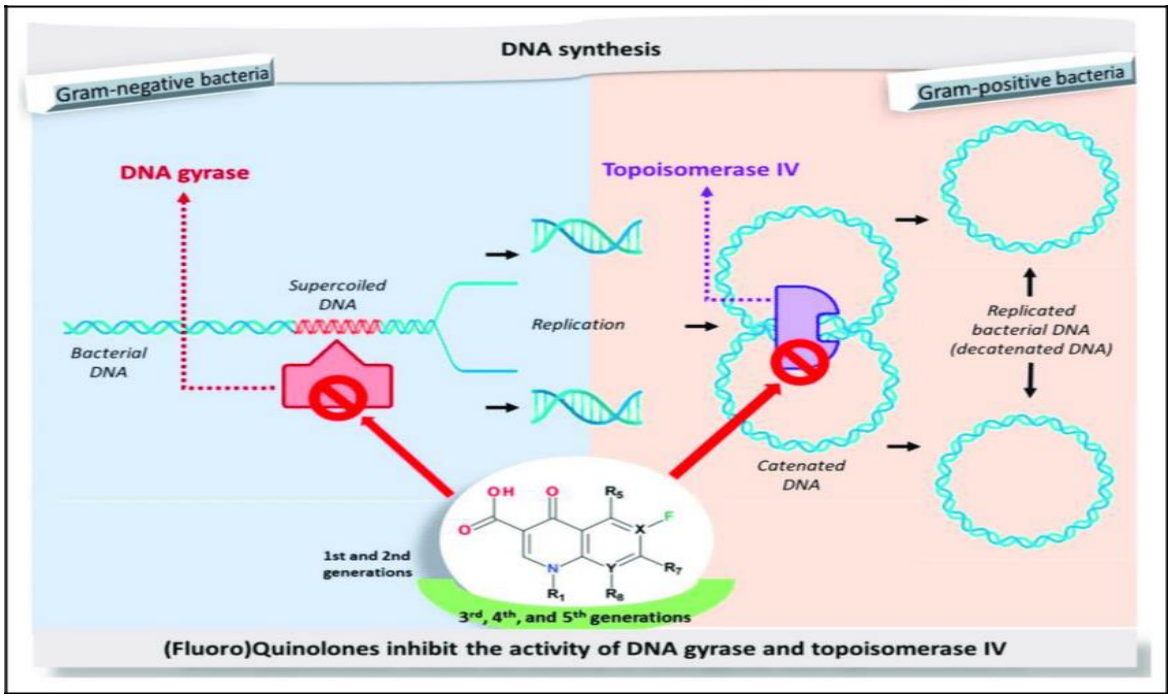


Figure 1: Mechanism of action of fluoroquinolones

The topoisomerase II enzyme allows the relaxation of supercoiled DNA by breaking both strands of DNA chain, crossing them over, and then combining them (Figure 1).Bacterial gyrase is different from mammalian topoisomerase so that quinolones and fluoroquinolones are 1000-fold more selective toward bacteria over the corresponding enzyme in humans. [25]

ANTI-VIRAL ACTIVITY OF FLUOROQUINOLONES

Fluoroquinolones are known to inhibit the replication of RNA viruses such as dengue, Zika, and hepatitis C viruses [26, 27, 28, 29, 30]. The antiviral mechanisms of action are not fully understood, but have been advised to include interference with viral entry and the inhibition of the viral helicase [26, 27, 29]. Several fluoroquinolones have been identified in large antiviral screens or molecular docking analyses as possibly interfering with the SARS-CoV-2 infectious cycle. Enoxacin and levofloxacin were identified in an in vitro screen as potential SARS-CoV-2 inhibitors with activityin the low micromolar range [31], while ciprofloxacin was predicted to interact with the SARS- CoV-2 main protease using in silico models [32, 33] . Depend on similarities in structure, activity,and safety profiles, moxifloxacin and levofloxacin were recommended as potential treatments to reduce SARS-CoV-2 respiratory symptoms [34]. As those drugs are FDA-approved, rearranging them as an antiviral therapy to fight the ongoing COVID-19 pandemic is an useful option. We analysed the potency of enoxacin, ciprofloxacin, levofloxacin, and moxifloxacin to suppress SARS-

CoV-2 and MERS-CoV in two cell types and found antiviral activity only at high micromolar concentrations. The cell types used in this were Vero cells, African green monkey kidney cells that are interferon-deficient [35, 36], and A549 human lung cells that have been developed to overexpress the SARS-CoV-2 entry receptor ACE2. It has been documented previously, fluoroquinolone antiviral potency likely varies by cell type and level of cellular differentiation [26, 28] .

Fluoroquinolones in HIV-AIDS

Fluoroquinolones are highly effective antibiotics with many advantageous pharmacokinetic properties. However, their extensive use has led to the development of antimicrobial resistance. Fluoroquinolones exhibit greater inhibitory activity against bacteria at higher concentrations, which helps in inhibiting bacterial growth. In the human body, when human lymphocyte CEM cell lines are infected with HIV-1, it results in cell death. However, fluroquinolones protect the infected cells from HIV-1-mediated cytolysis.

Some examples of fluoroquinolones include ciprofloxacin, norfloxacin, and enoxacin.Infection of human lymphocyte CEM cell lines with HIV-1(Human Immunodeficiency Virus type- 1, LAV-1 strain) leads to cell death. Ofloxacin, a fluoroquinolone antibiotic, has shown protective effects against HIV-1-mediated cytolysis in infected cells. Other fluoroquinolones such as ciprofloxacin, norfloxacin, and enoxacin also exhibit similar protection against HIV-1-mediated cytolysis. [37]

In the study of structure-activity relationships (SAR), it was found that the aryl substituents on the piperazine nitrogen play a crucial role in the anti-HIV-1 activity. Several compounds demonstrated potent anti-HIV activity, with an IC50 value of 0.06μM in chronically infected cells. The risk of repeated nontyphoid Salmonella (NTS) bacteraemia and the trends of antimicrobial resistance in NTS among HIV infected patients receiving highly active antiretroviral therapy(HAART) are currently unknown. In conclusion, the risk of repetitive NTS bacteremia has significantly decreased in the HAART era. However, NTS isolates obtained from HIV infected patients are increasingly showing resistance to fluoroquinolones. Therapeutic drug monitoring of fluoroquinolones is beneficial in ensuring that maximum Cmax to MIC ratios are achieved, particularly in patients at risk for malabsorption, such as those infected with HIV.

Fluoroquinolones in hepatitis and influenza

Fluoroquinolones, such as ofloxacin, should be used as Firstline treatment for cystic fibrosis. Cyclophosphamide and all other drugs, except for prednisolone, which was increased to 20mg daily, were stopped. Prolonged hepatitis is rare, and the Committee on Safety of Medicines received 18 reports of liver disorders out of a total of 640 reports of adverse reactions to ofloxacin (personal communication with the Regional Drug and Therapeutic Committee). There have been a few case reports implicating ofloxacin in the induction of hepatitis among quinolones.

Hepatitis C is a common cause of chronic liver disease. Fluoroquinolones are effective antimicrobial agents in the treatment of conditions associated with liver failure, including portal systemic encephalopathy (PSE). They have also been reported to possess antiviral properties against both DNA and RNA viruses, including HCV [38].

Fluoroquinolones in Covid-19

FQs were indicated to be active against certain viruses in cultured mammalian cells. Ofloxacin had inhibitory activity against the vaccinia virus (VV) and it prevents the pox tail lesion formation in mice infected with VV. [39] However, FQ derivatives inhibited the replication of HIV-1, HIV-2 [40] , and African swine. [41] The efficacy of some FQs e.g. ciprofloxacin was reported for Hepatitis C Virus (HCV) and the BKpolyomavirus (BKV) [42, 43], Ofloxacin was active against rhinovirus (RV). [44

Mechanism for antiviral activity

Among the identified drug target for coronaviruses is the main protease (Mpro, or 3CLpro) [45] and papain-like protease(s) PLpro), which are essential contributors in the viral replication cycle. These enzymes are not available in human cells so that drugs targeting these enzymes will be specific with a minor impact on human cells. Silico studies demonstrated that FQ (e.g. moxifloxacin), binds to and inhibits SARS-CoV-2 Mpro, consequently prevent its replication.[46]

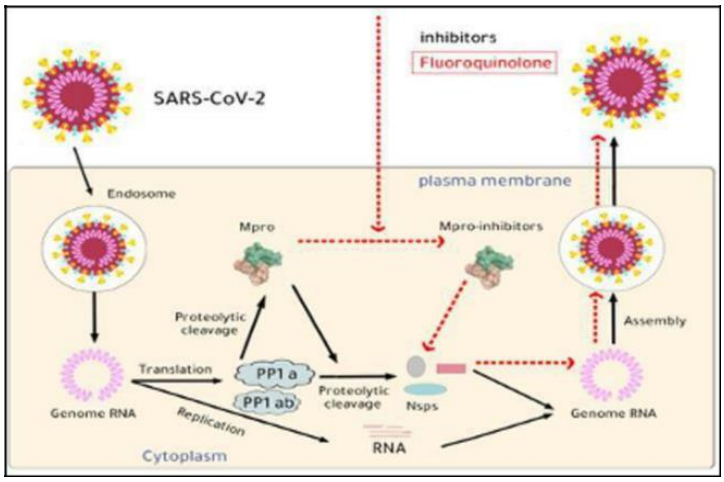


Figure 2. Proposed Cellular Effect of FQ on SARS-CoV-2

ANTI-TUMOR ACTIVITY OF FLOUROQUINOLONES

In the study,the type II Topoisomerase enzymes are essential for the formation of required DNA during the cellular processes such as replication, transcription, folding, and unfolding of DNA. [52, 53, 54]The existence of DNA Topoisomerases in both mammalian and bacterial cells makes them a good target for antibacterial and antitumor developing drugs [55,56]Mammalian Topoisomerases represent a potential target for antitumor drug design. For example, camptothecin and doxorubicin are anticancer drugs that target Topo I and Topo II enzymes, respectively [57, 58]. The quinolone drugs act by inhibition of Topoisomerases II in both prokaryotic and eukaryotic cell, due to these similarities of the prokaryotic and eukaryotic type; many studies were performed to selectively shifting of quinolones from an antibacterial to an antitumor activity [59, 60, 61] .

Quinolones have many required characteristic properties as cancer therapy such as less toxic, low frequency of developing tumor resistance, and a lesser possibility for the development of drug- caused secondary tumors, as well as favorable physicochemical and pharmacokinetic properties [62].Voreloxin , was the first quinolones derivative approved by the FDA as therapy for acute myeloid leukemia [63,64]. Also, ciprofloxacin was reported as a Topo II inhibitor and antitumor activities[65] against different human cancer cell lines such as colorectal [66], leukemia [67], and osteosarcoma [68] human cancer cell lines. Moreover, the Topoisomerase inhibition and the tissue penetration of quinolones are greatly affected by the modification at C3 and C-7 positions, which transforms its selectively from bacterial to human Topoisomerase II [69]. Hence, new cytotoxic fluoroquinolone analogs can be developed through different modifications at 7 or 3 positions of quinolones scaffold leading to decreases zwitterion characters, hydrophilicity nature and simultaneously enhance the activity.

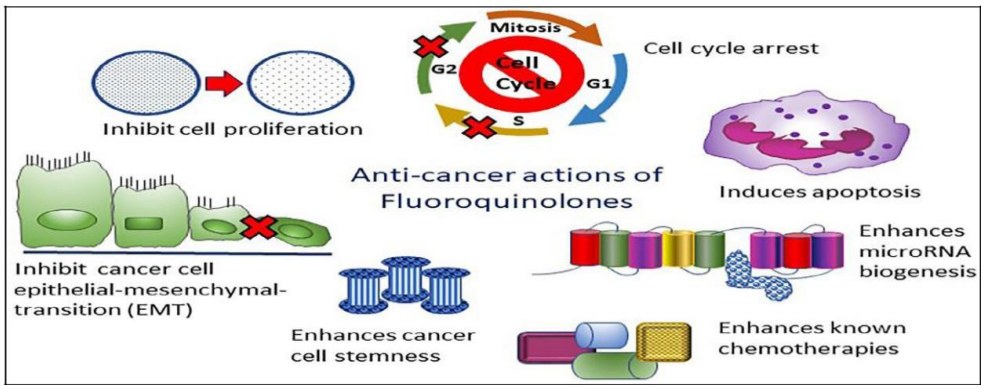


Figure 3 Anti-cancer activities of the Fluoroquinolones

The type II topoisomerases are the enzymes that essential for maintaining and controlling the configurations required for DNA replication,transcription, packaging and uncoiling of DNA in chromatin [70,71,72,73] . Like bacterial cells, eukaryotic species require a type II topoisomerase for viability [74]. The presence of DNA topoisomerases in both eukaryotic and prokaryotic cells makes them an target for chemotherapeutic intervention in antibacterial and anticancer therapies [75,76]. Mammalian topoisomerases represent potential target for several clinically useful anticancer drugs. For example, camptothecin is anticancer drug that targets type I topoisomerases that prevent religation of cleaved DNA strands and trigger apoptosis prefereably in dividing cells [77]. Type II topoisomerases are also effective cellular target for anticancer drugs such as doxorubicin. These drugs disturb the catalytic function of type II topoisomerases by trapping the enzyme in complex with cleaved DNA , preventing the process of relegation, by enhancing the formation of cleavable complexes or by inserting DNA, preventing the enzyme from performing its catalytic function .

Due to the mechanistic similarities exhibited by the prokaryotic type II topoisomerases (DNA gyrase and topoisomerase IV) and the eukaryotic type II topoisomerases, uncertain efforts to shift selectively quinolones from an antibacterial to antitumor agents were made by synthesizing new derivatives of quinolones [75]. New reports have determined the importance of fluoroquinolones as antiproliferative agents [72] via inhibition of DNA topoisomerase II as primary target. Ciprofloxacin was reported to inhibit topoisomerase II in eukaryotic, including mammalian cells . The inhibition of DNA topoisomerases and the cell permeability of quinolones are greatly influenced by the nature of the C-7 substituent . Introduction of an aryl moiety at C-7 position of quinolone transforms its selectivity from bacterial to human topoisomerase II

.On the other hand, research studies have shown the ability of fluoroquinolones to induce apoptosis and cell cycle arrest in different cancer cell lines either alone or in combination with other chemotherapeutic agents. It is reported that at a concentration exceeding 80 µg/ml ciprofloxacin was found to induce apoptosis, while at concentration of 25 µg/ml, it inhibits proliferation of Jurkat cells without cell death.

However, ciprofloxacin and moxifloxacin were found to induce G2 cell cycle arrest and apoptosis of different cancer cells. Also, gatifloxacin was found to induce S and G2-phase cell cycle arrest in pancreatic cancer cells *via* p21/p27/p53 pathway . On the other hand, literature study reported that fluoroquinolones, due to their expected effect on eukaryotic topoisomerase, can induce mutagenic properties in mammalian cells. Another study revealed that inhibition of this enzyme can be involved in the genotoxicity of fluoroquinolones at effective concentration.

Mechanism for Anti-tumor activity

Quinolones act by blocking the normal process of DNA topo I and II enzymes (especially, II) leading to inhibit the DNA synthesis. Topoisomerase enzymes have been performed essential functions during cell life by keeping the proper DNA topology intact.They manage the DNA supercoiling levels during the process of unwinding and rewinding of DNA double helix that's happened during the process of replication, recombination, repairing, and cell division. Topoisomerase enzymes have induced the formation of DNA breaks followed by religation after the passage of the other DNA strands through these breaks .

Quinolones drugs can affect the activity of topoisomerase enzymes either by inducing the formation of DNA breaks or stabilization of a transient DNA break that formed by a covalent compound with the enzyme, through which strand passage can occur and keep it from going back to the original DNA or through intercalation of DNA leading to inhibit the cell division and induce apoptosis. Topoisomerase II enzyme represents the target for quinolone derivatives as antimicrobial and anticancer agents. Moreover, certain quinolones exhibited anticancer activity by targeting Topoisomerases I and II enzymes.In addition, some quinolones exhibited antiproliferative activites the different mechanisms such as protein kinases CK2 inhibition or antimitotic activity or different cell cycle arrest pathways. Several anticancer drugs, known as Topoisomerase I & II inhibitors, for example, camptothecin[58] and its silicon-including analog,Karenitecin as topo I inhibitors.Also,etoposide and doxorubin are topo II inhibitors.

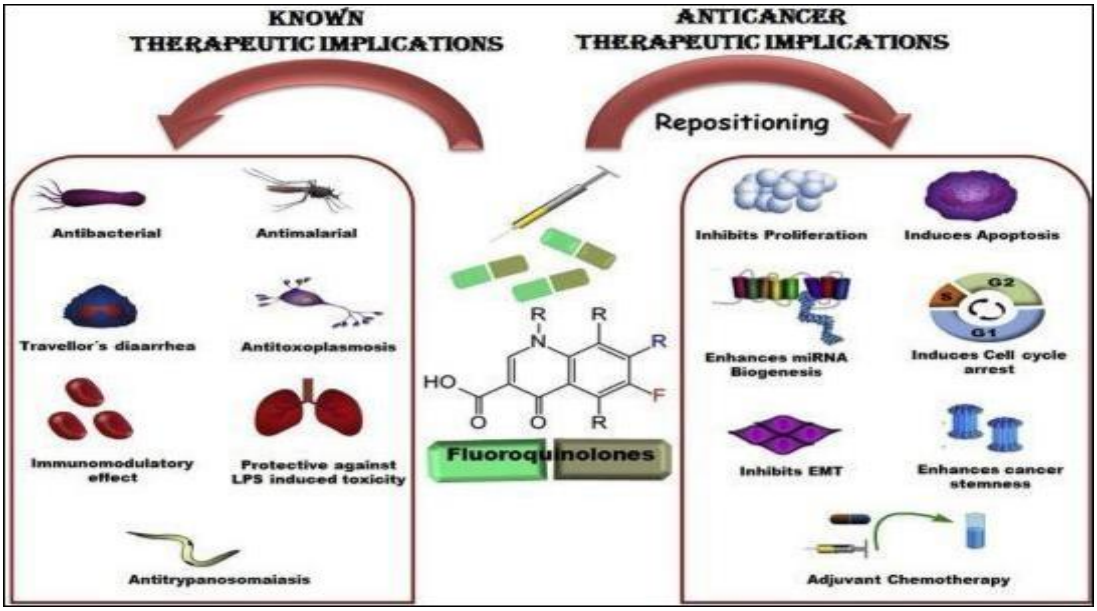


Figure 4 Repositioning of Fluoroquinolones as anti-cancer agents

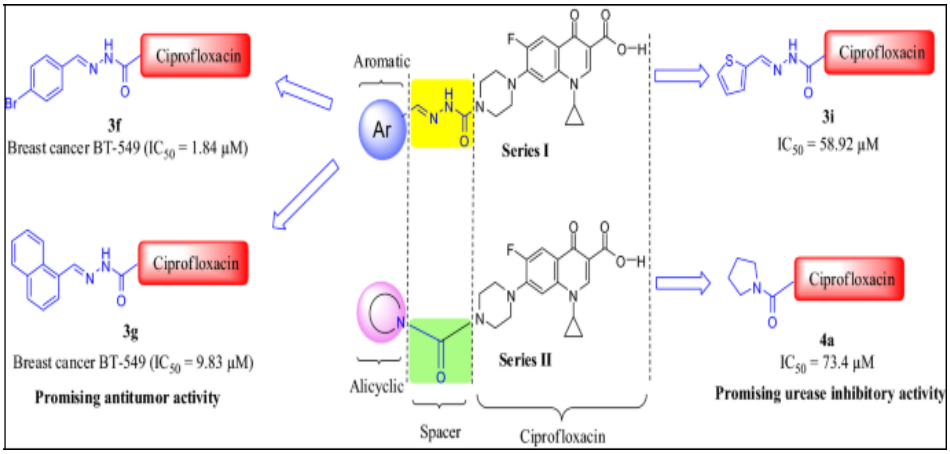
ANTI-FUNGAL AND UREASE INHIBITORY ACTIVITY OF FLUOROQUINOLONES

Antifungal Activitity

Few quinolone derivatives such as the fourth generation drugs gatifloxacin and moxifloxacin in addition to some norfloxacin derivatives such as compound were found to possess a significant antifungal activity . Some quinolone drugs as ciprofloxacin and moxifloxacin showed a synergistic activity to antifungal drugs, and was found to be useful for treatment of cases with associated bacterial and fungal infections .

Urease Inhibitory Activity

The fluoroquinolones, levofloxacin and ciprofloxacin were found to have superb ability to inhibit urease enzyme with excellent activity against Proteus mirabilis. Also, these study revealed that the Urease inhibitory activity was mediated *via* binding with the two Ni ions of the active site of Helibacter pylori. As a result, levofloxacin and ciprofloxacin could be used as urease inhibitors to prevent the formation of kidney stones associated with urinary tract infection .It was reported that norfloxacin can inhibit urease activity in Proteus Vulgaris and Proteus mirabilis utilizing sub- inhibitory concentrations .



In Vitro Synergistic Effect of Fluoroquinolone analogues in combination with artemisinin against Plasmodium falciparum; their antiplasmodial action in rodent malaria model

Drug-resistant malaria has emerged as the most undefiable obstacle in the battle against this deadly disease[1,2]. Artemisinin and its analogues, once regarded as the most powerful drugs that cure chloroquine-resistant Plasmodium falciparum infections, have also fallen to resistance [3-6]. Therefore, the need of the hour is to ward off the deployment of artemisinin and its analogues as monotherapy, to support WHO's resolution of advocating artemisinin-based combination therapy (ACT),and ensure their methodical and practicable implementation in all afflicted areas. As the available ACT is only a handful, there is tremendous possibility of the selection of Plasmodium strains with acquired resistance towards them. Therefore, the current focus should be directed towards devising alternative ACT. The underlying mechanism behind the therapeutic effect of artemisinin based combinations is that the artemisinin component rapidly and effectively wipes out most of the parasites,while those that remain are successively annihilated by a high concentration of the partner drug [7]. The first quinolone identified to possess activity against multiple parasite forms was endochin, 4(1H)-quinolone compound, in avian malaria models [14].A long time after its discovery back in the 1940s, other fluoroquinolones, such as norfloxacin, ciprofloxacin, pefloxacin, grepafloxacin, trovafloxacin, enoxacin, and clinafloxacin were evaluated against the malaria parasite in vitro [9,15,16] and in vivo [17-19]. Although these common antibiotics were found efficacious against both chloroquine-sensitive and -resistant parasites, highly effective concentrations and prolonged treatment regimen(14 days) have restricted their use as sole therapy. These findings support further screening of newer fluoroquinolone compounds as partner drugs.

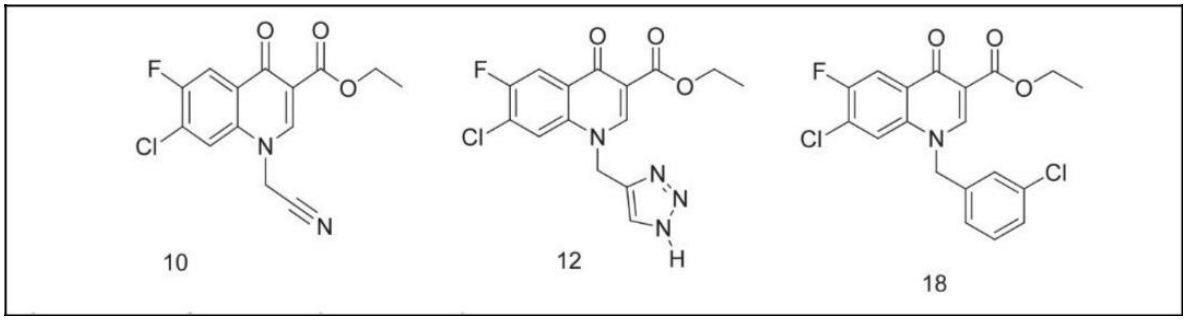


Figure 6 Chemical structures of compounds 10, 12 and 18

Therefore, it was considered interesting to investigate the fixed-ratio combinatorial interactions of each of these three novel fluoroquinolone derivatives with artemisinin, for treating the erythrocytic stages of P. falciparum strain 3D7. A modified isobologram method [21]was followed to assess the synergistic, antagonistic or additive interactions of the combinations. Additionally, on account of their convincing antiplasmodial activity under in vitro conditions, it was imperative to assess their efficacy in vivo, employing a rodent malaria model.

Methods

Ant plasmodial interaction assay of artemisinin and fluoroquinolone analogue combinations

Parasite culture

Stock culture of malaria parasite P. falciparum 3D7 strain (chloroquine sensitive) was continuously maintained in vitro using a CO2 incubator under low-oxygen concentration(3%) and high carbon dioxide atmosphere (4%) along with nitrogen (93%), incubated at a temperature of 37°C. The parasites were maintained on O+ human red blood cells suspended in a complete culture medium. Each litre of RPMI-1640 aqueous culture medium was prepared with 10.4 g of powdered RPMI-1640 (with glutamine but without sodium bicarbonate), 5.94 g of HEPES buffer, 1 g of dextrose and 40 mg of gentamicin. Complete medium was constituted just before use by adding sterile 5% sodium bicarbonate at the rate of 4 ml per 96 ml, and supplemented with 10% (v/v) pooled O+ human serum. Infected erythrocytes were suspended in this culture media initiated at a haematocrit value of 5% and parasitemia was kept between 2 and 4% with sub-culturing done beyond 5%. Medium was changed once a day and percentage parasitemia was monitored using Giemsa stained slides.

Stock solution of compounds

Artemisinin (Sigma Aldrich, USA) was prepared in DMSO to get the stock solution of 1 mg/ml strength.Compounds 10, 12 and 18 were synthesized according to the procedure described by Dixit et al. [20] and each compound was made to strength of 1 mg/ml stock solution in DMSO. The stock solutions were diluted on the day of experiment to get the desired concentrations for each compound. The highest amount of DMSO in diluted concentrations was 0.125%, and had no effect on parasite growth.

Preparation of fixed-ratio combinations

In each combination assay, two compounds (Compound A, artemisinin and Compound B, a fluoroquinolone derivative or norfloxacin) were combined in four fixed ratios (4:1, 3:2, 2:3, and 1:4). Approximately eight-fold IC50 compound concentration of the respective compound A or B was taken as 100% (calculated to be 32 nM, 69.50 µM,30.34 µM, 55.40 µM, and 430.88 µM for artemisinin, compound 10, compound 12, compound 18 and norfloxacin, respectively), so that IC50 of the individual compound falls in between third and fourth two-fold serial dilution.

Isobologram preparation and data analysis

For each combination assay, IC50 was calculated from two sets of concentration response graphs, each containing compound alone curve and four combination curves. The sum FIC of each combination ratio of two combined compounds shows that the drug-drug schizontocidal interaction between them [22] was determined by the following equation:

$$\text{Sum FIC}(\sum \text{FIC}) = \frac{\text{IC50 of A in mixture}}{\text{IC5O of A alone}} + \frac{\text{IC5O of B in mixture}}{\text{IC50 of B alone}}$$

ΣFIC <1 represents synergism, ΣFIC > = 1 and <2 represents additive interaction, ΣFIC > = 2 and <4 represents slight antagonism while ΣFIC > = 4 represents marked antagonism[23-25]. Mean FICs of the combinations were compared by frequency distribution using GraphPad Prism 5, to define if a compound was superior to the other one, when combined with artemisinin.

In vivo efficacy of fluoroquinolone analogues using rodent malaria model

Evaluation of the curative potential of the

Plate preparation for anti-plasmodial interaction assay

Compound dilutions of each combination solution were made in sterile, flat-bottomed, 96-well tissue culture plates as described by Fivelman et al. [21]. Six times two-fold serial dilution was done for each combination in triplicate.Each well contained a total volume of 200 µl of complete culture medium with or without compound and presynchronized infected red blood cells (1% parasitemia at 2.5% haematocrit). Control cultures (without compound) were maintained on the same plate in triplicate. Two 96-well plates (for six combinations) were used for each combination experiment. The plates were stacked in a CO2 incubator and incubated at 37°C for 48 hours.

Slide preparation, staining and assessment

After 48-hour incubation, thin blood smear slides were prepared, air dried, methanol fixed, and stained in Giemsa solution for 40 min. After staining, slides were removed from coupling jar, washed in running tap water and air dried. The Giemsa-stained slides were examined for counting the number of parasites in random adjacent microscopic fields, equivalent to about 4,000 erythrocytes at 1,000 × magnification. Per cent parasitemia was calculated. Reproducibility of counts was checked by two other readers to maintain the quality control. fluoroquinolone derivatives was done using the method described by Ryley and Peters, 1970 (rodent malaria four-day suppressive test; Peters' four-day suppressive test) [26,27]. Rodent malaria parasite Plasmodium berghei ANKA was used.

Experimental animals

Immuno-compromised BALB/c inbred albino mice (25–30 g) of the male sex were obtained from the Animal Facility Centre of the Department of Zoology, University of Delhi. The animals were fed ad libitum with standard feed and had free access to water. They were maintained under standard conditions of humidity, temperature (25°C) and 12 hours light/darkness cycles. The animals were acclimatized for two weeks before the commencement of the study and were ensured to exclude all zoonotic agents.

Test procedure

Day 0: Heparinized blood was withdrawn from an infected donor mouse with approximately 25-30% parasitemia, and diluted in 1x PBS to 108 parasitized erythrocytes per mL. An aliquot of 0.2 mL (=2 × 107 parasitized erythrocytes) of this suspension was injected intraperitoneally (ip) into experimental

groups of five mice each. One to three hours post-infection, the experimental groups were treated with varying doses of each of the test compounds (0.5, 1, 10,25 mg/kg BW) by the ip route. Each compound was made to strength of 5 mg/ml stock solution in 10% DMSO and administered according to desired concentration and individual body weight. Artemisinin was given to the standard drug group and 0.2 mL of normal saline to the negative control group.

Day 1, 2 and 3: 24 hours, 48 hours and 72 hours postinfection, the experimental groups of mice were treated again with the same dose and by the same route as on day 0.

Day 4: 24 hours after the last treatment (i.e., 96 hours post-infection), blood was drawn from the tail region of mice and smears were prepared. These were stained with Giemsa for microscopic analysis by counting four fields of approximately 500 erythrocytes per slide, for five replicates of each sample, to determine the parasitemia percentage and hence assess the anti-malarial efficacy of the test compounds. Differences in parasitemia percentage between treated groups and untreated animals were analysed by a one-way ANOVA test using IBM SPSS Statistics 16.0 and differences considered significant if $P < 0.05$. Furthermore,the difference between the mean value of the negative control group (taken as 100%) and those of the experimental groups was calculated and expressed as percent inhibition (= activity) using the equation below and hence ED50 value was calculated graphically.

Per cent inhibition (activity) = 100 – Mean parasitemia/control- 100

Untreated control mice typically died approximately one week after infection. Treated mice were observed for a period of 30 days, and the survival time (in days) was recorded. The mean survival time was calculated in comparison to untreated (Normal saline) and standard drug (artemisinin) treated groups. Differences in survival time between treated groups and untreated animals were analysed by Log-rank (Mantel-Cox) test using GraphPad Prism 5 and differences considered significant if $P < 0.005$.Observations concerning adverse effects due to the compounds were recorded.

LD50 Test: LD50 test was carried out on BALB/c mice using different dosages of various

compounds: 50, 100, 200, 500,600, 800 and 1000 mg/kg BW ip and the animals were observed

for 7 days. Therapeutic Index (TI) values were determined by the

formula:Therapeutic Index(TI) = $\frac{\text{Median Lethal Dose(LD50)}}{\text{Median Effective Dose(ED50)}}$

RESULTS:

Antiplasmodial interactions between artemisinin and fluoroquinolone analogues

Various substituted fluoroquinolones were synthesized by the earlier reported procedure [20]. The pharmacophore of fluoroquinolones shows striking similarity with chloroquine, which has been the forerunner for malaria

Table 2 Interaction between artemisinin and various fluoroquinolones analogs (compounds 10, 12 and 18) against Plasmodium falciparum (3D7 strain) at six different preparations

Combination solution Ratio (A:B)	Drug A (Artemisinin) Mean FIC50 ± Sea	Drug B (Compound-10) Mean FIC50 ± Sea	Σ FICs, interactionb
1 (5:0)	1.02 ± 0.04	0	
2 (4:1)	0.61 ± 0.01	0.15 ± 0.02	0.76 SYN
3 (3:2)	0.58 ± 0.03	0.22 ± 0.02	0.80 SYN
4 (2:3)	0.48 ± 0.02	0.53 ± 0.03	1.01 ADD
5 (1:4)	0.17 ± 0.03	0.41 ± 0.03	0.58 SYN
6 (0:5)	0	0.93 ± 0.04	
Combination solution Ratio (A:B)	**Drug A (Artemisinin) Mean FIC50 ± Sea**	**Drug B (Compound-12) Mean FIC50 ± Sea**	**Σ FICs, interactionb**
1 (5:0)	1.0 ± 0.02	0	
2 (4:1)	0.63 ± 0.03	0.15 ± 0.003	0.78 SYN
3 (3:2)	0.60 ± 0.03	0.23 ± 0.02	0.83 SYN
4 (2:3)	0.50 ± 0.02	0.77 ± 0.03	1.27 ADD
5 (1:4)	0.25 ± 0.004	0.32 ± 0.01	0.56 SYN
6 (0:5)	0	1.11 ± 0.08	

Combination solution Ratio (A:B)	Drug A (Artemisinin) Mean FIC50 ± Sea	Drug B (Compound-18) Mean FIC50 ± SEa	Σ FICs, interactionb
1 (5:0)	0.97 ± 0.07	0	
2 (4:1)	0.58 ± 0.02	0.16 ± 0.01	0.73SYN
3 (3:2)	0.55 ± 0.03	0.47 ± 0.03	1.02ADD
4(2:3)	0.49 ± 0.04	0.66 ± 0.03	1.15ADD
5 (1:4)	0.22 ± 0.01	0.33 ± 0.02	0.55SYN
6 (0:5)	0	0.97 ± 0.08	

Combination solution Ratio (A:B)	Drug A (Artemisinin) Mean FIC50 ± Sea	Drug B (Norfloxacin) Mean FIC50 ± SEa	Σ FICs, interactionb
1 (5:0)	1.13 ± 0.06	0	
2 (4:1)	0.91 ± 0.03	0.45 ± 0.02	1.36 ADD
3 (3:2)	0.29 ± 0.02	0.52 ± 0.04	0.81 SYN
4 (2:3)	0.66 ± 0.02	0.87 ± 0.03	1.53 ADD
5 (1:4)	0.14 ± 0.01	0.60 ± 0.02	0.74 SYN
6 (0:5)	0	0.98 ± 0.04	

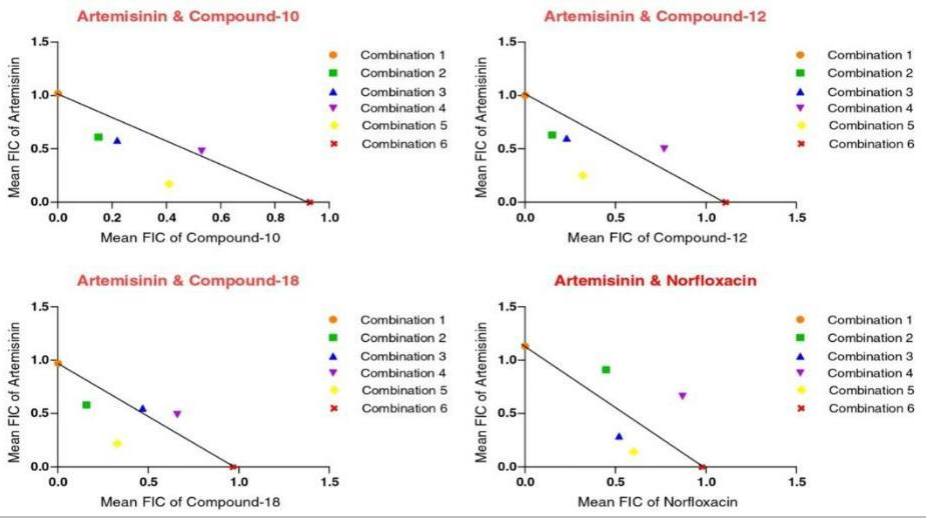


Figure 7: Isobolograms showing interaction between artemisinin and fluoroquinolone derivatives; artemisinin and norfloxacin against Plasmodium falciparum 3D7 strain.

treatment for the past 50 years [28]. Both fluoroquinolones and chloroquine contain chlorine at position 7. A vast array of fluoroquinolones have been investigated and henceforth proven effective against P. falciparum. Here, three substituted fluoroquinolone compounds, 10, 12 and 18 were chosen from previous in vitro study[20], and were tested in combination with artemisinin in vitro against P. falciparum chloroquine-sensitive 3D7 strain, using norfloxacin as the standard drug. These compounds were found to have synergistic and additive drug-drug interactions. In every combination assay IC50 was determined from two sets of drug response curves obtained from each replicate, each set representing four combination curves and a curve of drug/compound alone. Mean FIC50 values derived from these curves are tabulated in Table 2, for each fluoroquinolone derivative combination with artemisinin, and combination of norfloxacin with artemisinin. Sum of FICs are presented in isobolograms (Figure 7). The isobolograms show that anti-malarial interaction of the fluoroquinolone derivatives in vitro with artemisinin is not antagonistic. Compound 10 in combination with artemisinin shows synergistic antiplasmodial interaction in three of the four fixed-ratio combinations evaluated and additive in the remaining one. Similarly the combination of compound 12 and artemisinin displays synergistic interaction in three combinations and additive in one.

Table 3 Antiplasmodial activity of fluoroquinolone derivatives against Plasmodium berghei strain ANKA

Compound	**Mean ED50 (mg/kg BW) ± SEa**
10	2.31 ± 0.19
12	3.09 ± 0.22
18	2.60 ± 0.18
Artemisinin	1.72 ± 0.15

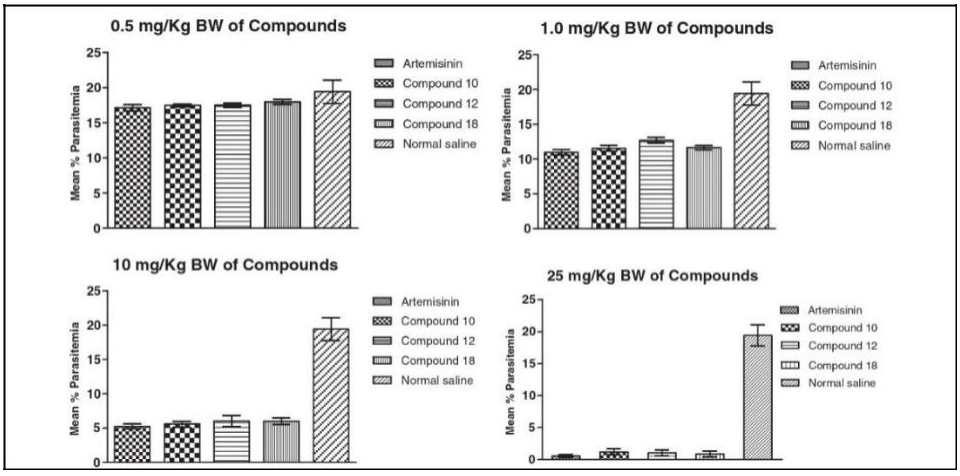


Figure 8 Effects of various compounds and artemisinin on established P. berghei infections in mice. The experimental hosts were infected on day 0 and treated intraperitoneally with normal saline; compounds 10, 12, 18 and artemisinin at 0.5, 1.0, 10, or 25 mg · Kg−1 BW · day−1 on days 0 to 3, as described by Ryley and Peters. Data expressed as Mean ± SD of five mice per condition.

Interaction of artemisinin with compound 18 was found to be synergistic in two combinations, while additive in the other two. The standard drug used in the study, norfloxacin, when combined with artemisinin shows synergistic interaction in two combinations, while additive in the remaining two. Combination of compound 10 with artemisinin tended most towards synergism, with respect to all the other compounds (Mean ΣFIC ± SD = 0.788 ± 0.177), as observed by frequency distribution. Combinations of all fluoroquinolone analogues were superior to that of norfloxacin (Mean ΣFIC ± SD = 1.11 ± 0.394), which tended slightly towards antagonism. In principle, components of anti-malarial combinations should target different metabolic pathways. This condition is being theoretically met in the combinations being evaluated. The hypothesized mechanisms of action of artemisinin include haem alkylation, inhibition of PfATP6 (SERCA-type enzyme), parasite membrane damage [29-31]. On the contrary, fluoroquinolones are the only class of antimicrobial agents that are direct inhibitors of bacterial DNA synthesis. They inhibit two bacterial enzymes: DNA gyrase, particularly the A subunit, and topoisomerase IV, which have essential roles in DNA replication [32]. Plasmodium falciparum contains a functional apoplast, an organelle of prokaryotic origin. The 27–35 kb circular genome of apoplast requires bacterial type DNA gyrase for its duplication [33-35]. This is the most likely explanation for inhibitory activity of fluoroquinolones against the parasite,which is being enhanced on interaction with artemisinin either synergistically or additively, analysed using fixed ratio is bolograms. However, the exact mode of action of fluoroquinolones against malaria parasites is still ambiguous. Antiplasmodial activity of synthetic fluoroquinolones against Plasmodium berghei in vivo.It was observed that there was a reduction in the levels of parasitemia in all the test groups, as well as that of the standard drug (artemisinin) group. However, the reverse was the case for the negative control group, as there was a marked increase in parasitemia level. The in vivo anti-malarial activity of the various test compounds, after conducting Peters' four-day suppressive test, is presented in Figure 8. Results were significant as analysed by ANOVA ($P < 0.05$). The ED50 values were calculated to be 2.31, 3.09, 2.60, and 1.72 mg/kg BW for each of the compounds 10, 12, 18, and artemisinin, respectively, as indicated in Table 3 and represented graphically in Figure 9.

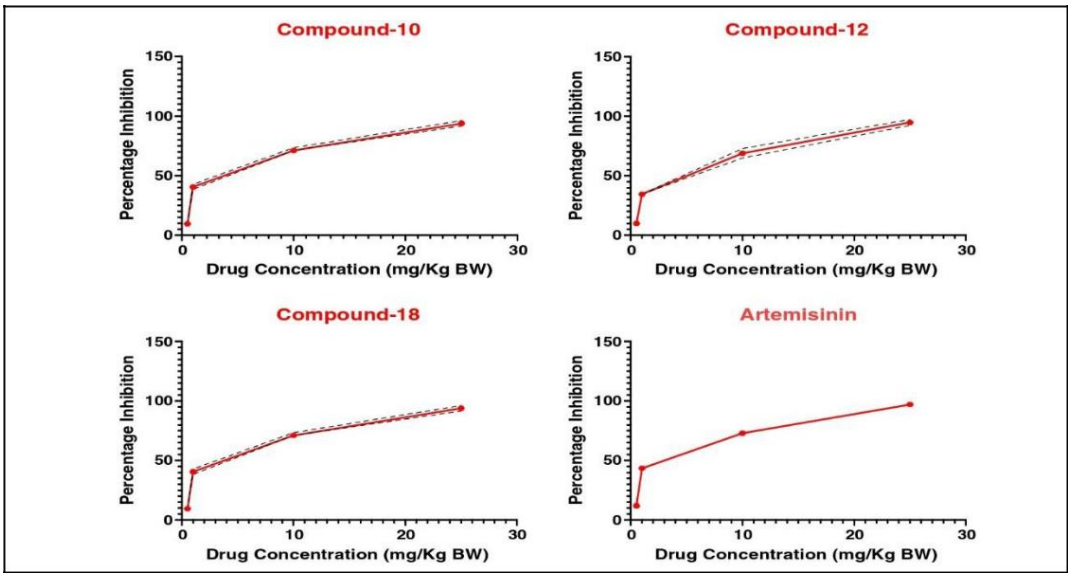


Figure 9 Dose–response curves of fluoroquinolone derivatives and artemisinin, against in vivo blood stages of Plasmodium berghei strain ANKA. Data expressed as Mean ± SD of five mice/compound.

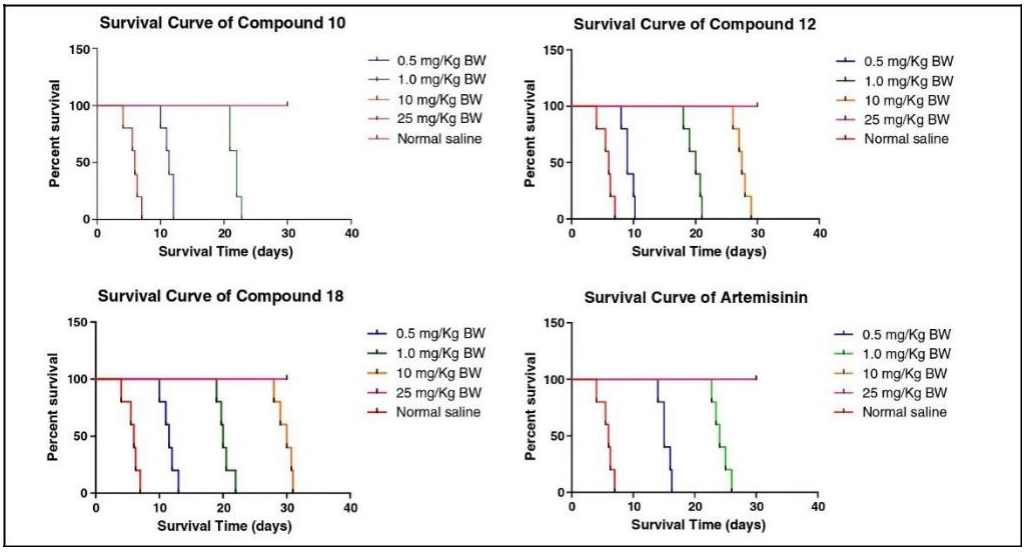


Figure 10 Kaplan Meier survival analysis curves of BALB/c mice, administered drugs once daily ip for four consecutive days (5 mice per group). Results between test and control were significant by P < 0.005 as analysed by Log-rank test.

The mean survival time (MST)values of the treated groups were significantly higher than that of control and were comparable to that of the standard drug, artemisinin (Figure 5). The mice treated with varying doses of each of the fluoroquinolone derivatives survived beyond one week, but 0.5 mg/kg BW treated mice died nine to 12 days post treatment. Artemisinin treated mice on the other hand, survived beyond two weeks in all the groups. The results show that two out of the three compounds (10 and 18) exhibited appreciable antiplasmodial activity, reflected by their ED50 values, comparable to that of the standard drug, artemisinin. The study on MST demonstrated a dose-dependent increase in the number of days the mice survived in various groups survived post four-day treatment. MST values for compound 10 (with lowest ED50 = 2.31 mg/kg BW) were the most proximal to those obtained for artemisinin, while the next to follow was compound 18 (ED50 = 2.60 mg/kg BW). All the results were significant (P < 0.005) as analysed by Log rank(Mantel-Cox) test.

Therefore, they serve as promising candidates for further research. No significant adverse side effects, i.e., physical signs such as gasping for air, loss of appetite, feeling sleepy, or weight loss were observed in compound-treated groups, even at the highest dose administered, indicating that compounds 10 and 18 could be excellent candidates for combination therapy.

LD50 Test :

BALB/c mice died at 1000 mg/kg BW of all the compounds and could tolerate 500 mg/kg BW. However, at 800 mg/kg BW, half the population of mice died. Therapeutic indices were determined as 346.32, 258.90, 307.69 for compounds 10, 12 and 18, respectively.

CONCLUSION:

We conclude that the new strategies of multitargeting and repositioning of fluoroquinolones from antibacterial to anti-tumour or to anti-viral agents as well as the new strategies for developing new antibacterial fluoroquinolones with improved properties. In fact,the modified quinolones are

synthesized have permitted definition of the basic chemical requirements for improving activity against viruses The mechanism of action of antiviral quinolone is unclear, most of these drugs were tested and proved to be effective against HIV-1, but when assayed against a variety of different viruses, quinolones were shown to possess non-specific activity. These data indicate that drug's targets are likely to be structures common to a wide range of viruses.

The advent of quinolones as antiviral agents is particularly attractive since quinolones are extremely versatile small organic molecules. Finally the

development of drugs with novel targets to be used in combination with clinically available antiretroviral agents is particularly attractive to the enhance effectiveness and reduce undesired side effects and the development of resistance towards the drugs. The growing resistance to fluoroquinolones and the need for broad spectrum potent analogues the development of fluoroquinolone derivatives. This gives the chance for invention of new drugs with enhanced activity or properties, especially those with better pharmacokinetic profile and effective against resistant virulent strains or anaerobic organisms. It is obvious that quinolones have captured the interest of researcher in the past 50 years. In the future years, researchers aim to utilize quinolone scaffold in discovering optimal antibiotic.

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