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Quinolone Compounds with Activity Against Multidrug-Resistant Gram-Positive Microorganisms

Pintilie Lucia

Additional information is available at the end of the chapter

Abstract

The emergence of resistance to antimicrobial agents is a global public health problem. Some microorganisms may develop resistance to a single antimicrobial agent (or related class of agent), while others develop resistance to several antimicrobial agents or classes. These organisms are often referred to as multidrug-resistant or MDR strains. Identification of new molecules that show activity against multidrug-resistant microorganisms and its development on a new antimicrobial drug, would be an important step in the fight against antimicrobial resistance. This paper presents experimental data regarding the synthesis of several quinolones. The novel compounds having quinolone structure were synthesized by Gould-Jacobs method. Their structure has been determined and confirmed by the following physicochemical methods: elemental analysis, IR spectral analysis, H-NMR, C-NMR, UV, thin layer chromatography. The new compounds have been evaluated for „in vitro" activity by determining minimum inhibitory concentration against a variety of bacteria Some of new quinolones, which showed a good activity, have been tested against 30 strains of methicillin resistant Staphylococcus aureus isolated in the Microbiology Laboratory of INBI Prof. "Dr. Matei Bals" during 2012 The minimum inhibitory concentration (MIC) of the isolates have been determined by agar plate Mueller Hinton (bioMerieux) dilution method using the reference strain Staphylococcus aureus ATCC 29213. The 30 strains of isolated have been also tested for susceptibility to ciprofloxacin, levofloxacin and imipenem by Etest method. Base on the "in vitro" studies, the quinolone FPQ-30 appears to be an promising compound, all strains isolates were inhibited at a concentration of 8 μg/ml.

Keywords: Quinolones, fluoroquinolones, antimicrobial agents, Methicillin-resistant Sthaphilococcus aureus

1. Introduction

The emergence of resistance to antimicrobial agents is a global public health problem. Some microorganisms may develop resistance to a single antimicrobial agent (or a related class of agents), while others develop resistance to several antimicrobial agents or classes. These organisms are often referred to as multidrug-resistant or MDR strains. Identification of new molecules that show activity against multidrug-resistant microorganisms and its development into a new antimicrobial drug would be an important step in the fight against antimicrobial resistance.

The discovery of fluoroquinolones after 1980 represented a decisive step forward for chemical anti-infectious therapy. A large number of fluoroquinolones are used today in medical practices and some of them are deemed by leading pharmacologists to be of vital importance to anti-infectious therapy.

2. Tendencies and strategies in the field of quinolones

The basic structure of quinolones (Figure 1) [9], is a bicyclic structure that contains a ring type A 4-pyridinone combined with an aromatic or heteroaromatic ring B. According to the nature of atoms symbolized by X, Y, Z, they can be defined as four subfamilies: naphthyridine 1-8, cinnoline, pyrido-2,3-pyrimidines, and quinolone.
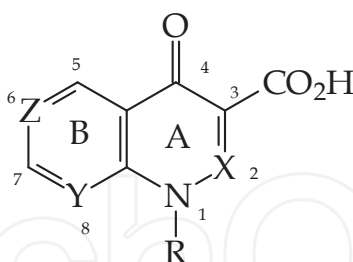
naphthyridines : X=Z=H; Y=N
cinnolin: X=N; Y=Z=H
pyrido-2,3-pyrimidine: X=H; Y=Z=N
quinolones : X=Y=Z= H

Figure 1. Basic structure of quinolones.

The structural modifications of the core of the quinolone influence the antimicrobial activity

2.1. Position 1

Research has been oriented in several directions:

- Introducing an unsubstituted or substituted alkyl: R_1 = methyl [32], ethyl [32, 28], *iso*propyl [28, 48, 1], *tert*-butyl [48, 14], fluoroethyl [28], hydroxyethyl [38], chloroethyl [38];

- Introduction of a vinyl, allyl [38, 32];

- Introduction of alkylamino groups [65];

- Introduction of a cyclpopropyl [38, 56, 57, 49] or cyclobutyl [49, 1];

- Introducing mono or disubstituted phenyl [38, 7, 46, 49];

- Introduction of a five-membered aromatic heterocycles: pyrrolyl, [34].

Usually, the most active compounds contain the ethyl substituent in position 1. Using a STERIMOL program, Fujita (1984), based on a set of five parameters that characterize the shape and size of a substituent and following a quantitative analysis of the relationship between chemical structure and biological activity on a set of N-1 allyl and alkyl derivatives have deduced the optimum length of the substituent in position 1 is 0.42 nm [9, 58], which corresponds to the ethyl substituent. In general, the most active components contain in position 1 an ethyl substituent. These studies have already been confirmed by the results achieved with quinolones that are used in therapeutic practice: nalidixic acid, oxolinic acid, norfloxacin, pefloxacin, quinolones that have an ethyl substituent in N-1 position.

In Table 1 is presented the antimicrobial activity for the 1-substituted quinolones against gram positive and gram negative microorganisms. By comparison, data are entered for norfloxacin (R_1 = ethyl) and ciprofloxacin (R_1 = c-C_3H_5).

Replacing ethyl substituent with:

- Sterically comparable substituents (2-fluoroethyl and vinyl) lead to compounds with comparable activity on gram negative bacteria, while replacing with substituent steric hindrance effect more or less pronounced (methyl, 2 hydroxy-ethyl, n-propyl, allyl, benzyl, cyclopropyl-methyl) leads to decreased activity of "in vitro".

- *Tert*-butyl substituent leads to compounds that possess good antimicrobial activity against *Staphylococcus aureus*, while against gram negative microorganisms, the activity is comparable to the reference compound (norfloxacin). Increasing the number of carbon atoms (number of carbon atoms > 4) results in decreased antimicrobial activity. The replacement of a hydrogen by fluorine atom leads to the increase of antibacterial activity two to three times compared to the compound where R_1 = *tert*-butyl, while introducing more fluorine atoms leads to a decrease in the antimicrobial activity.

In series N-1 cyclopropyl (ciprofloxacin), the substituent from N-1 position has a larger volume than the ethyl. This is contrary to the concept of the steric volume. High antimicrobial activity of the ciprofloxacin may be caused by this hyperconjugation or self-association properties induced by the cyclopropyl. For compounds that present in this position, a cycloalkyl with a number of carbon atoms of more than 3 (cyclobutyl, cyclopentyl) the antimicrobial activity is much reduced compared to ciprofloxacin.

The following are relevant for the chemical structure–biological activity relationships:

- The nature of the substituent introduced in the alkyl moiety at N-1 position of quinolone: the fluorine atom has benefic influence on the antimicrobial activity;

- The position where the substituents are introduced in the alkyl moiety: substituents in position 1 of the alkyl moiety have positive influence on antimicrobial activity;

- The number of substituents placed on the alkyl moiety: increasing the number of substituents leads to decreased antimicrobial activity;

- Presence of cis-trans stereoisomers: cis derivatives are more active against gram positive bacteria than the trans derivatives, while the activity is comparable against gram negative bacteria.

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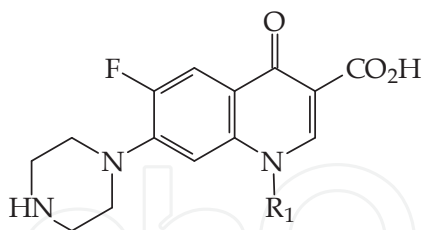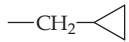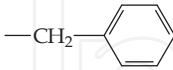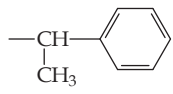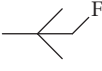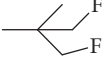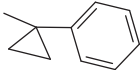

Figure 2. Quinolones with an aliphatic substituent in N-1 position.

R$_1$	Minimum inhibitory concentration (µg/ml)			Reference
	S. aureus	*E. coli*	*P. aeruginosa*	
-CH$_3$	6.25	0.39	1.56	(Koga et al. 1980)
-C$_2$H$_5$ (Norfloxacin)	0.39	0.05	0.39	(Koga et al. 1980)
-CH=CH$_2$	3.13	0.10	0.39	(Koga et al. 1980)
n-C$_3$H$_7$	1.56	0.20	3.13	(Koga et al. 1980)

R$_1$	Minimum inhibitory concentration (μg/ml)			Reference
	S. aureus	E. coli	P. aeruginosa	
—CH₂—◁ (cyclopropylmethyl)	1.00	0.50	4.00	(Bouzard et al. 1989)
—CH₂—phenyl (benzyl)	1.56	0.78	1.56	(Koga et al. 1980) (Bouzard et al. 1989)
isopropyl	1.00	0.50	1.00	(Bouzard et al. 1989)
isopropenyl	0.50	0.13	0.50	(Bouzard et al. 1989)
—CH(CH₃)—phenyl	4.00	2.00	32.00	(Bouzard et al. 1989)
tert-butyl	0.25	0.06	0.50	(Remuzon et al. 1991)
sec-butyl	0.25	0.50	4.00	(Bouzard et al. 1989)
fluoro-tert-butyl (F)	0.13	0.016	0.25	(Remuzon et al. 1991)
difluoro-tert-butyl (F, F)	1.00	0.13	1.00	(Remuzon et al. 1991)
-CH₂CH₂F	1.56	0.10	0,.78	(Koga et al. 1980)
-CH₂CH₂OH	1.56	0.39	3.13	(Koga et al. 1980)
-NH-CH₃	1.95	1.00	1.00	(Koga et al. 1980) (Wentland et al. 1984)
-CH₂-CH=CH₂	3.13	0.20	1.56	(Koga et al. 1980)
cyclopropyl (Ciprofloxacin)	0.13	0.03	0.013	(Chu&Fernandes 1991)
phenylcyclopropyl	0.13	0.13	4.00	(Bouzard et al. 1989)
methylcyclopropyl	0.25	0.06	0.50	(Bouzard et al. 1989)
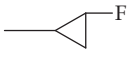
R₁	Minimum inhibitory concentration (µg/ml)			Reference
	S. aureus	*E. coli*	*P. aeruginosa*	
(trans)	1.00	0.06	2.00	(Bouzard et al. 1989)
(cis)	0.13	0.13	1.00	(Bouzard et al. 1989)
	1.00	1.00	32.00	(Bouzard et al. 1989)
(cis)	0.10	≤0.05	≤0.05	(Youichi et al. 1994) (Shongo et al. 1993)
(trans)	0.78	≤0.05	0.10	(Youichi et al. 1994) (Shongo et al. 1993)
	0.50	0.13	1.00	(Bouzard et al. 1989)
	4.00	2.00	32.00	(Bouzard et al. 1989)
	0.50	0.25	8.00	(Bouzard et al. 1989)
	2.00	1.00	8.00	(Bouzard et al. 1989)
	8.00	2.00	63.00	(Bouzard et al. 1989)

Table 1. MIC values (µg/ml) for quinolones with an aliphatic substituent in N-1 position

It is obvious that the volume of the substituent at position N-1 is not the only factor influencing the antibacterial activity of the quinolones. There are other factors that have a considerable influence on biological activity such as, for example, the effects of conjugation, conformational effects. This has been demonstrated by the synthesis of compounds containing aryl substituents in N-1 position [32, 7, 8, 46].

In Table 2 are presented data on the antimicrobial activity of 7-piperazinyl and 7- (4-methyl-piperazinyl)-quinolone derivatives having substituted or unsubstituted aryl substituents in N-1 position.
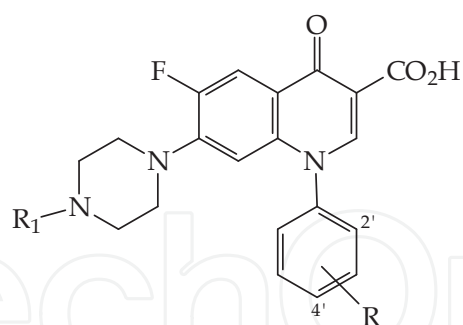
Figure 3. Aryl-quinolones.

Compound	R	R_1	Minimum inhibitory concentration (μg/ ml)			Reference
			S. areus	*E. coli*	*P. aeruginosa*	
	Norfloxacin		0.39	0.05	0.39	2
1	H	H	0.20	0.78	0.78	(Chu et al. 1985)
Sarafloxacin	4-F	H	0.20	0.05	0.39	(Chu et al. 1985)
2	H	CH_3	0.78	0.78	6.20	(Chu et al. 1985)
3	2-F	CH_3	1.56	0.78	6.20	(Chu et al. 1985)
4	3-F	CH_3	12.50	6.20	50.00	(Chu et al. 1985)
Difloxacin	4-F	CH_3	0.20	0.20	1.56	(Chu et al. 1985)
5	4-Br	CH_3	3.10	6.20	50.00	(Chu et al. 1985)
6	4-Cl	CH_3	1.56	1.56	12.50	(Chu et al. 1985)
7	4-OH	CH_3	0.05	0.10	0.39	(Chu et al. 1985)
8	4-OCH_3	CH_3	1.,50	50.00	200.00	(Chu et al. 1985)
9	4-CH_3	CH_3	1.56	1.56	12.50	(Chu et al. 1985)
10	3,4-OCH_2O-	CH_3	0.75	0.78	6.20	(Chu et al. 1985)
11	2-F,4v-F	CH_3	0.10	0.20	1.56	(Chu et al. 1985)
12	2′-CH_3	CH_3	3.10	1.56	25.00	(Chu et al. 1985)
13	2-CH_3,4CH_3	CH_3	100.00	100.00	100.00	(Chu et al. 1985)
14	4-NO_2	CH_3	64.00	8.00	128.00	(Radl & Zikan 1989)
15	4-NH_2	CH_3	2.00	1.00	32.00	(Radl & Zikan 1989)

Table 2. The "in vitro"" antibacterial activity of 1-aryl-quinolones

For *7-piperazinyl quinolones*, the introduction of an unsubstituted aryl affords a compound (compound 1 – Table 2) with good antimicrobial activity "in vitro" against *S. aureus* being more active than the reference compound, norfloxacin.

The presence of a substituent on the aryl nucleus leads to improved antimicrobial activity, e.g. a fluorine atom (sarafloxacina) in the 4-position of the phenyl ring.

In the series 7-(4-methyl-piperazinyl)-1-aryl-quinolones the best results are obtained when in position 4 is located a fluorine atom (for example, difloxacin) or -OH (compound 7). Good results are also obtained in the case of two substituents present (positions 2 and 4) on the aryl nucleus (R = F) (compound 11).

2.2. Position 2

Some changes were explored for this position [9]. Replacing the carbon atom of oxolinic acid with a nitrogen atom (which led to cinoxacin) has resulted in improved pharmacokinetic properties, but has led to decreased antimicrobial activity "in vitro". Significant reductions in bacterial activity were also observed in the case of 2-aza-4-quinolones derivatives from norfloxacin and pefloxacin. The introduction of substituents on the carbon atom in position 2, for example, hydroxy, methyl, methylthio, etc., leads to the inactivation of the quinolone compounds.

2.3. Position 3

In the quinolone molecule, combination between carboxyl group from 3 position and ketone group in 4 position, is considered necessary for binding to DNA gyrasa, while the presence of carboxyl group in 3 position is essential for antimicrobial activity. Modification of carboxyl groups, generally leads to obtaining biologically inactive compounds (Figure 4) [62]. The exceptions are groups that can be converted "in vitro" to the carboxyl function.
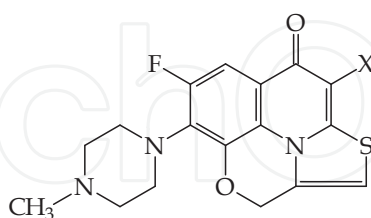
Figure 4. X = H, 4b: X = SO_3H

The researches regarding changes of the substituent in the 3 position of quinolone, followed to obtain:

- Compounds presenting free carboxyl group;

- Bioreversible compounds (prodrugs) ester (Figures 5, 6), [59, 51], which easily hydrolyzed in the body releasing the carboxyl group;
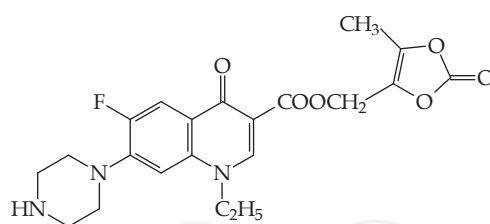
Figure 5. Methyl-2-oxo-1,3-dioxol-4-yl) ethyl ester
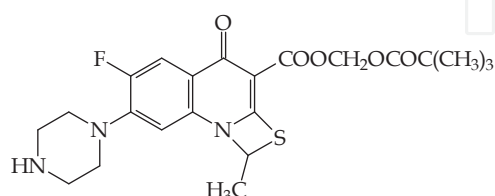
Figure 6. Pivaloiloxymethyl ester
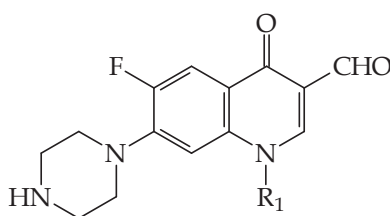
Figure 7. R_1 = ethyl, cyclopropyl, R = H, methyl

- Bioreversible derivatives (prodrugs) - 3-formyl-quinolone (Figure 7) [31];

- Quinoline-3-carboxamide: (Wetland et al. 1993), (Sajay et al. 2000).
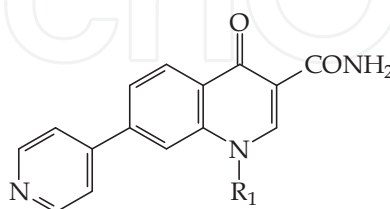
Figure 8. R_1 = alkyl, mono or disubstituted phenyl

Both types of structures (Figures 8 and 9) have been hydrolyzed in the body; carboxyl group has been generated; and through this the biological activity was explained, with the observation that the time to reach that MICs is greater.
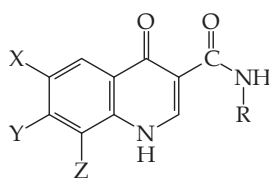
Figure 9. X = H,Cl,F,NO$_2$, Y = H,Cl,NO$_2$ Z = H,CH$_3$,F, R = cyclopropyl, n-octyl, (N, N-diethylamino-1-methyl) butyl

2.4. Position 4

The quinolone structure requires the presence of the ketone group in position 4. Replacing this ketone group, the 4-thiooxo or 4-sulfonyl groups led to obtaining inactive compounds [9].

2.5. Position 5

In position 5 of the quinolones various groups were introduced, such as nitro (Domagala et al. 1988) unsubstituted or substituted amino [27, 16, 36] [17], alkyl [23, 25], halogen [35, 36], mercapto [35, 36], hydroxy [36, 17], alkoxy or tioalcooxi [35, 36]
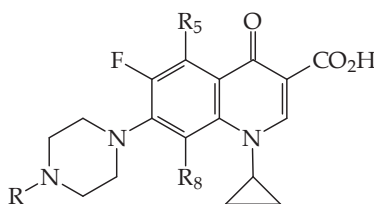
Figure 10. Substituted quinolones.

R	R$_5$	R$_8$	Minimum inhibitory concentration (μg/ml)			Reference
			S. aureus	*E. coli*	*P. aeruginosa*	
H	H	H	0.2	0.013	0.4	(Domagala et al.1991)(*)
H	NH$_2$	H	0.025	0.013	0.8	(Domagala et al.1991)(*)
H	NH$_2$	F	0.013	0.013	0.025	(Domagala et al.1991)(*)
			0.013	0.013	0.025	(Domagala et al.1988)(*)
			0.050	0.0063	0.1	(Myamoto et al. 1990) 74(**)
H	CH$_3$NH	F	0.2	0.1	1.6	(Domagala et al.1988)(*)
H	AcNH	F	>25	>25	>25	(Domagala et al.1988)(*)
H	NH$_2$	Cl	0.025	0.025	0.8	(Domagala et al.1991)(*)
H	HO	H	0.2	0.025	0.4	(Domagala et al.1991)(*)

R	R_5	R_8	Minimum inhibitory concentration (µg/ml)			Reference
			S. aureus	E. coli	P. aeruginosa	
H	HO	F	0.2 0.2	0.05 0.025	0.8 0.39	(Myamoto et al. 1990) (**)
H	CH_3	H	0.025	0.013	0.2	(Hagen et al. 1991) (*)
H	CH_3	F	0.025	0.025	0.2	(Hagen et al. 1991) (*)
H	CH_3	Cl	0.025	0.025	0.2	(Hagen et al. 1991) (*)
H	C_2H_5	H	6.30	0.4	12.5	(Hagen et al. 1991) (*)
H	F	F	0.2	0.0125	0.39	(Myamoto et al. 1990) (**)
H	Cl	F	0.1	0.0125	0.39	(Myamoto et al. 1990) (**)
CH_3	H	F	0.2	0.025	0.39	(Myamoto et al. 1990) (**)
CH_3	F	F	0.39	0.05	0.78	(Myamoto et al. 1990) (**)
CH_3	Cl	F	0.2	0.025	0.78	(Myamoto et al. 1990) (**)
CH_3	Br	F	0.39	0.05	0.78	(Myamoto et al. 1990) (**)
CH_3	HO	F	0.2	0.05	0.78	(Myamoto et al. 1990) (**)
CH_3	CH_3O	F	25	0.2	12.5	(Myamoto et al. 1990) (**)
CH_3	$PhCH_2O$	F	6.25	1.56	6.25	(Myamoto et al. 1990) (**)
CH_3	HS	F	3.13	0.39	12.5	(Myamoto et al. 1990) (**)
CH_3	CH_3S	F	3.13	0.2	12.5	(Myamoto et al. 1990) (**)
CH_3	$4-CH_3OPhCH_2S$	F	100	12.5	>100	(Myamoto et al. 1990) (**)
CH_3	NH_2	F	0.1 0.013	0,0125 0.025	0.2 0.1	(Domagala et al.1988)(*) (Myamoto et al. 1990) (**)
CH_3	CH_3NH	F	0.78	0.1	1.56	(Myamoto et al. 1990) (**)
CH_3	$(CH_3)_2N$	F	25 >25	3,.3 >25	50 >25	(Domagala et al.1988) (Myamoto et al. 1990) (**)
CH_3	$PhCH_2NH$	F	0.78	0.78	3.13	(Myamoto et al. 1990) (**)
CH_3	$HOCH_2CH_2NH$	F	0.39	0.5	0.78	(Myamoto et al. 1990) (**)
CH_3	$(CH_3)_2NCH_2CH_2NH$	F	6.25	0.05	0.78	(Myamoto et al. 1990) (**)
CH_3	pyrrolyl	F	3.13	0.39	12.5	(Myamoto et al. 1990) (**)

Ciprofloxacina ; * S. aureus UC 76 ; * E. coli Vogel; * P. aeruginosa.UI-18; **S. aureus 209P JC-1 ; **E. coli NIIHJ JC-2; **P. aeriginosa 12

Table 3. The "in vitro" antibacterial activity of 5-substituted quinolones

In the series of *1-cyclopropyl-7-piperazinyl quinolones*, the influence of the substituent in position 5 on the antimicrobial activity is manifested as follows:

- The introduction of unsubstituted amino group when $R_8 = H$ leads to increased antibacterial activity against gram positive only, while when $R_8 = F$ or Cl leads to the increase of antibacterial activity on gram positive and gram negative microorganisms.

- The introduction of substituted amino group leads to the lowering of antimicrobial activity on the entire microbial spectrum.

- The introduction of the hydroxy group ($R_8 = H, F$), does not produce any change in the antibacterial activity.

- The introduction of the methyl group ($R_8 = H, F, Cl$) causes increased activity on the entire microbial spectrum.

- The introduction of an alkyl radical having a carbon number greater than 2 leads to decreased antimicrobial activity.

- The introduction of a halogen atom:

- For $R_5 = F$ ($R_8 = F$) – the antimicrobial activity remains unchanged on the entire microbacterial spectrum.

- For $R_5 = F$ ($R_8 = F$) – antibacterial activity is improved against gram positive bacteria).

In the series of *1-cyclopropyl-7-(4-methyl-piperazinyl)-quinolones*, the influence of the substituent in position 5 on the antimicrobial activity is manifested as follows:

- The introduction of an unsubstituted amino group ($R_8 = F$) leads to increased antibacterial activity against gram positive and gram negative microorganisms; the alkylation of the amino group leads to the lowering of antimicrobial activity on the entire microbial spectrum, the decrease being dependent on the size of the alkyl group (especially dialkylating lead to a loss of antibacterial activity).

- The introduction of a halogen atom (fluorine or bromine) ($R_8 = F$) leads to the slight decrease of activity compared to the unsubstituted compound; the introduction of a chlorine atom decreases antibacterial activity only against *Pseudomonas aeruginosa.*

- The introduction of a hydroxy group does not modify the biological activity. The introduction of a methoxy or benzyloxy group leads to decreasing of the antimicrobial activity against all tested microorganisms.

- The introduction of a mercapto group leads to a considerable decrease of the antimicrobial activity on the entire microbial spectrum. The same decrease is found in the case of methylthio group.

- The presence of a bulky substituent leads to the loss of biological activity against all microorganisms tested.

2.5.1. In conclusion

In the case of 1-cyclopropyl-7-piperazinyl-quinolones, antibacterial activity increases according to the nature of the substituent in position 5, in the following order:

For R_8 = H : RO < HO < NH_2 < CH_3;

For R_8 = F : HO ≤ F ≤ Cl < CH_3 < F

In the case of 1-cyclopropyl-7-(4-methyl-piperazinyl)-8-fluoro-quinolones, antibacterial activity increases according to the nature of the substituent in position 5, in the following order:

R_2N < RH < SH < RO < RNH < Br F ≤ HO ≤ Cl < NH_2

2.6. Position 6

The nature of the substituent from this position influences the inhibition activity of DNA gyrase and cell penetration.

In this position the following substituents have been introduced: H, F, Cl, Br, CH_3, NO_2, NH_2 [32, 5, 7, 34, 29, 4, 5].

The introduction of a fluorine atom in this position has lead to a spectacular increase of the antibacterial activity, compared to unsubstituted compound (R_6 = H) (activity of norfloxacin against *E. coli* R_6 = F, is 16 times higher than the nonfluorinated compound in position 6) (Table 6).

Regarding the importance of the presence of fluorine atom in position 6 of the quinolone, other authors (Ledoussant et al. 1992) have reported that this is essential for the activity of the quinolone compounds.

R_6	Minimum inhibitory concentration (µg / ml)			Reference
	S. aureus	*E. coli*	*P. aeruginosa*	
H	12.50	0.78	3.13	(Koga et al. 1980)
F: Norfloxacina	0.39	0.05	0.39	(Koga et al. 1980)
Cl	1.56	0.20	3.13	(Koga et al. 1980)
Br	3.13	0.39	1.50	(Koga et al. 1980)
CH_3	3.13	0.39	6.25	(Koga et al. 1980)
SCH_3	25.00	0.78	12.50	(Koga et al. 1980)
$COCH_3$	100.00	100.00	100.00	(Koga et al. 1980)
CN	12.50	0.39	6.25	(Koga et al. 1980)
NO_2	25.00	0.78	12.50	(Koga et al. 1980)

Table 4. The "in vitro" antibacterial activity of 6 substituted quinolones
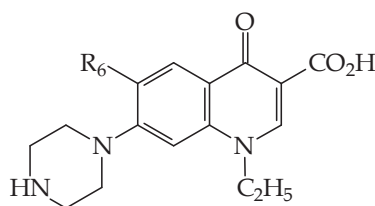
Figure 11. Substituted quinolones.

There have been synthesized compounds containing an amino group at position 6 [3-5]. A direct comparison between 6-amino-quinolones and 6-fluoro-quinolones shows a decrease of antimicrobial activity of 6-amino-quinolones of about 28–300 times (Table 7). The antimicrobial activity of 6-amino-quinolones can be improved by optimizing the chemical structure through the introduction of various substituents in other positions of the quinolones core.

	R_1	R_5	R_7	R_8	Minimum inhibitory concentration (µg/ml)			Reference
					S. aureus	*E. coli*	*P. aeruginosa*	
1 a	-c-C_3H_5	H	Piperazinyl	H	0.25	64.00	2.00	(Cecheti et al. 1995)
2 a	-c-C_3H5 *ciprofloxacin*	H	Piperazinyl	H	0.12	0.03	0.06	(Cecheti et al. 1995)
1 b	-c-C_3H_5	H	4-Methyl-piperazinil	H	0.25	2.00	4.00	(Cecheti et al. 1995)
1 c	-c-C_3H_5	H	Tiomorpholinyl	F	0.12	0.25	4.00	(Cecheti et al. 1995)
1 d	-c-C_3H_5	NH_2	4-Methyl-piperazinyl	H	128	16	128	(Cecheti et al. 1995)
1 e	-c-C_3H_5	NH_2	4-Methyl-piperazinyl	F	2	0.03	4	(Cecheti et al. 1995)
1 f	-c-C_3H_5	H	4-Methyl-piperazinyl	F	4	1	2	(Cecheti et al. 1995)
1 g	t-Bu	H	4-Methyl-piperazinyl	H	2	0.06	2	(Cecheti et al. 1995)
1 h	4-FC_6H_4	H	4-Methyl-piperazinyl	H	8	0.12	8	(Cecheti et al. 1995)

Table 5. The "in vitro" antibacterial activity of 6-amino-quinolones.
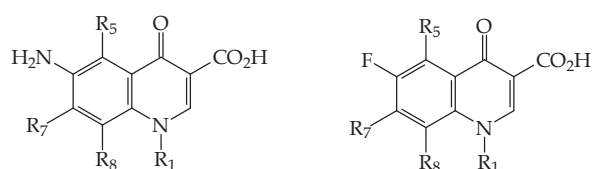
Figure 12. Amino-quinolones and 6-fluoro-quinolones.

2.7. Position 7

Modifications in position 7 have been the most intensively studied; studies have shown that the nature of the substituent from this position has a great influence on the biological potential, antibacterial spectrum, solubility, and on the pharmacokinetics (bioavailability). Research has been focused in the following areas: introduction of piperazinyl, mono or disubstituted piperazinyl, morpholinyl or thiomorpholinyl, pyrrolidinyl, piperidinyl, azetidinyl, bicyclic heterocycles, 4-pyridinyl.

Influence of the nature of the substituent in position 7 on the antibacterial activity is closely related to the nature of the substituents from the other positions of the quinolones core, especially the nature of the substituents at the nitrogen in position 1.

For *1-ethyl-6-fluoro-7-substituted-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid* (Table 6), it was observed that the best influence on antimicrobial activity is the introduction of a piperazinyl group (norfloxacin). Introducing the piperazinyl group in position 7 of the quinolone ring leads to a product with a higher antibacterial potential. The antibacterial spectrum of norfloxacin includes both gram positive and gram negative bacteria, in particular more strains of *P. aeruginosa*.
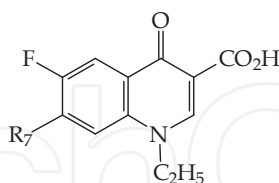
Figure 13. Ethyl-7-substituted-quinolones.

R$_7$	Minimum inhibitory concentration (µg/ml)			Reference
	S. aureus	*E. coli*	*P. aeruginosa*	
Chlor	12.50	1.56	100.00	(Koga et al.1980)
Methyl	6.25	0.39	5000	(Koga et al.1980)
Amino	100.00	3.13	100.00	(Koga et al.1980)

R$_7$	Minimum inhibitory concentration (µg/ml)			Reference
	S. aureus	E. coli	P. aeruginosa	
Methylamino	12.50	3.10	100.00	(Koga et al.1980)
Dimethylamino	0.78	0.39	50.00	(Koga et al.1980)
S(CH$_2$)$_2$NH$_2$	25.00	0.80	3.10	(Chu & Fernandes 1991)
Piperazinyl (norfloxacin)	039	0.05	0.39	(Koga et al.1980)
4-Methyl-piperazinyl	0.39	0.10	1.56	(Koga et al.1980)
4-Allyl-piperazinyl	0.39	0.39	6.25	(Koga et al.1980)
4-Benzyl-piperazinyl	0.39	0.79	50.00	(Koga et al.1980)
4- (4-Nitro-benzyl) -piperazinyl	1.56	6.25	100.00	(Koga et al.1980)
4- (4-Amino-benzyl) -piperazinyl	0.39	0.39	12.50	(Koga et al.1980)
4-Benzoyl-piperazinyl	1.56	3.13	25.00	(Koga et al.1980)
4- (2-Hydroxyethyl) piperazinyl	0.78	0.10	6.25	(Koga et al.1980)
3- (Hydroxymethyl) piperazinyl	8.00	2.00	64.00	(Ziegler et al. 1990)
3-Fluoromethyl-piperazinyl	0.12	0.12	32.00	(Ziegler et al. 1990)
3-Difluoromethyl-piperazinyl	0.12	0.50	32.00	(Ziegler et al. 1990)
Morpholinyl	0.78	0.20	12.50	(Koga et al.1980)
Thiomorpholinyl	0.06	1.60	12.50	(Chu & Fernandes 1991)
1-Piperidinyl	0.78	1.56	50.00	(Koga et al.1980)
4-Hydroxy-piperidinyl	0.39	0.39	6.25	(Koga et al.1980)
4-Dimethylamino-piperidinyl	0.39	0.10	3.13	(Koga et al.1980)
Pyrrolidinyl	0.20	0.39	12.50	(Koga et al.1980)
Pyrrolidyl	0.40	1.60	12.50	(Chu & Fernandes 1991)
Thiazolidinyl	0.20	0.20	3.10	(Chu & Fernandes 1991)
1-Imidazolyl	0.25	1.56	12.50	(Toshio et al. 1987)
1-Pyrazolyl	6.25	12.50	25.00	(Toshio et al. 1987)
1-Pyrrolyl	0.39	3.13	12.50	(Toshio et al. 1987)
1,2,4-Triazol-4-yl	25.00	25.00	25.00	(Toshio et al. 1987)
3-Amino-3-methyl-1-azetidinyl	0.50	0.50	2.00	(Toshio et al. 1987)

Table 6. The "in vitro" antibacterial activity of 1-ethyl-7-substituted-quinolones.

For *1-cycloprpyl-6-fluoro-7-substituted-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid* (Table 7) it was observed:

- Introducing a piperazinyl moiety (ciprofloxacin) yields a compound with a higher antibacterial potential; a similar antibacterial activity is obtained in the case where R_7 is 3-methyl-piperazinyl (Compound 1B);

- Introducing the pyrrolidinyl moiety (4A-4E compounds) gives a good bacterial activity on the entire bacterial spectrum, especially in the case of compound 4A where R_7 = 3-amino-pyrrolidinyl;

- Introducing an azetidinyl moiety (Compound 8A) leads to an approximately twofold increase in activity against gram positive bacteria compared to ciprofloxacin; activity against gram negative microorganisms is 4-15 times lower. Introduction of a substituent on the azetidinyl moiety offers compounds with increased activity in the following order: 8I(aminomethyl) ≈ 8J(thia) < 8D(ethylamino) < 8E(dimethylamino) < 8C(methylamino) < 8B(amino). For the 7-(3,3-di-azetidinyl)-quinolone, antibacterial activity increases in the following order: 8K (aminomethyl) ≈ 8L (ethylaminomethyl) < 8H (dimethylamino) < 8C (methylamino) < 8F (amino). In conclusion, the activity "in vitro" of the compounds 8B and 8F increases on the entire bacterial spectrum and can be compared with that of ciprofloxacin. For compounds where R_7 are 3-amino-2-methyl-azetidinyl (compounds 9A–9H), the effect given by the stereochemistry of the substituents in the azetidinyl moiety has been studied. The activity "in vitro" of the compound trans-7-(3-amino-2-methyl-azetidinyl) quinolone 9A can be compared favorably with the activity of 3-monosubstituted-azetidinyl, 3,3-disubstituted-azetidinyl and ciprofloxacin.
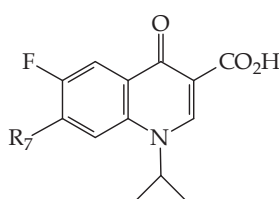
Figure 14. Cyclopropyl-7-substituted-quinolones.

Comp.	R$_7$	Minimum inhibitory concentration (µg/ml)			Reference
		S. aureus	*E. coli*	*P. aeruginosa*	
1A	Piperazinyl (ciprofloxacin)	0.13	0.03	0.13	(Bouzard et al. 1989)
1B	3-Methyl-piperazinyl	0.12	0.015	0.25	(Ziegler et al. 1990)
1C	3-Fluoromethyl-piperazinyl	0.12	0.06	2.00	
2A	Morpholinyl	0.05	0.20	1.56	(Kazico et al. 1993)
2B	3-Aminomethyl-morpholinyl	0.20	0.20	1.56	

Comp.	R$_7$	Minimum inhibitory concentration (µg/ml)			Reference
		S. aureus	E. coli	P. aeruginosa	
3A	Homopiperazinyl	0.25	0.03	1.00	(Ziegler et al. 1990)
3B	3-Hydroxy-homopiperazinyl	4.00	0.25	2.00	
3C	3-Fluoro-homopiperazinyl	0.25	0.03	2.00	
4A	3-Amino-pyrrolidinyl	0.025	0.025	0.1-0.2	(Domagala et al.1993)
4B	3-Aminomethyl-pyrrolidinyl	0.006	0.05	0.80	
4C	3-Methylamino-pyrrolidinyl	0.025	0.10	0.80	
4D	3-Thia-pyrrolidinyl	0.013	0.20	1.6	
4E	3-Aminomethyl-3-methyl-pyrrolidinyl	0013	0.20	0.80	
5	3-Hydroxy-pyrrolidinyl	<0.015	<0.015	0.125	(Petersen & Grohe 1992)
6	3-Oxo-pyrrolidinyl	1.56	6.20	100.00	(Cooper et al. 1992)
7A	3-Amino-4-trans-cyclopropyl-pyrrolidinyl	<0.025	0.05	0.80	(Bush et al. 1993)
7B	3-Amino-4-trans-(trans-2-CO$_2$Et)-cyclopropyl- pyrrolidinyl	0.20	0.20	3.10	
8A	Azetidinyl	0.06	0.12	2.00	
8B	3-Amino-azetidinyl	0.25	0.03	0.25	
8C	3-Methylamino-azetidinyl	0.25	0.03	0.50	
8D	3-Ethylamino-azetidinyl	0.25	0.06	2.00	
8E	3-Dimethylamino-azetidinyl	0.12	0.06	2.00	
8F	3-Methyl-3-amino-azetidinyl	0.12	0.06	0.50	(Frigola et al. 1993)
8G	3-Methylamino-3-methyl-azetidinyl	0.25	0.06	2.00	
8H	3-Dimethylamino-3-methyl-azetidinyl	0.25	0.25	4.00	
8I	3-Aminoethyl-azetidinyl	0.50	0.12	1.00	
8J	3-Thia-azetidinyl	0.50	0.25	4.00	
8K	3-Aminomethyl-3-methyl-azetidinyl	0.25	0.12	1.00	
8L	3-Thia-3-methyl-azetidinyl	0.25	0.12	4.00	
9					(Frigola et al. 1994)
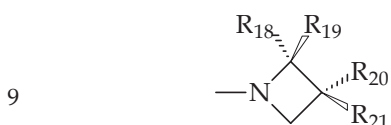
R$_{18}$	R$_{19}$	R$_{20}$	R$_{21}$

Comp.	R$_7$				Minimum inhibitory concentration (μg/ml)			Reference
					S. aureus	E. coli	P. aeruginosa	
9A	CH$_3$	H	H	NH$_2$	0,12.	0.03	0.25	
9B	CH$_3$	H	NH$_2$	H	0.12	0.06	0.50	
9C	C$_2$H$_5$	H	NH$_2$	H	0.25	0.12	2.00	
9D	H	CH$_3$	NH$_2$	CH$_3$	0.25	0.06	2.00	
9E	H	CH$_3$	NHCH$_3$	H	0.12	0.06	0.50	
9F	H	CH$_3$	N(CH$_3$)$_2$	H	0.25	0.50	4.00	
9G	H	CH$_3$	CH$_2$NH$_2$	H	0.25	0.12	2.00	
9H	H	CH$_3$	CH$_2$NHC$_2$H$_5$	H	0.25	0.12	4.00	

Table 7. The "in vitro" antibacterial activity of 1-cyclopropyl-7-substituted-quinolones.

For *1-(p-fluorophenyl)-7-substituted-quinolones* (Table 8), it is observed that the introduction of substituents: 3-methyl-piperazinyl (4), 4-amino-piperazinyl (8), 3 amino-pyrrolidinyl) (17), and 3-methyl-2-amino-pyrrolidinyl (20) has the effect of extending of the antibacterial spectrum on both gram positive and gram negative bacteria, while introducing morpholinyl groups (10), thiomorpholinyl (11), 4-hydroxy-piperidinyl (13), pyrrolidinyl (15), 3-hydroxy-pyrrolidinyl (compound 16) leads to the increase of antibacterial activity against gram positive bacteria, comparable with the antibacterial activity of the sarafloxacin and difloxacin. It should be noted that the introduction of the methyl group in position 5 of 3-amino-pyrrolidinyl substituent (compound 17) leads to obtaining a compound (20) with a high solubility in water and with excellent efficacy in oral administration.
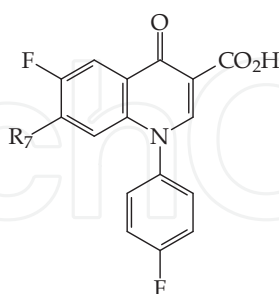
Figure 15. *p*-Fluorophenyl)-7-substituted-quinolones.

Structural changes were focused on replacing the nitrogen atom of the heterocyclic substituent with a sp^2 or sp^3 hybridized carbon atom. All new compounds have a high activity both "in vitro" and "in vivo". The potential of these compounds is relatively dependent on the size of the ring and the hybridization of the carbon atom through which it connects with the quino-lone. 1-Piperazino moiety can be substituted with 4-piperidinyl (compound 1, Table 9) with

	R$_7$	Minimum inhibitory concentration (µg/ml)			Reference
		S. aureus	*E. coli*	*P. aeruginosa*	
1	Piperazinyl (sarafloxacina)	0.20	0.05	0.39	(Chu et al. 1985)
2	4-Methyl-piperazinyl (difloxacin)	0.20	0.20	1.56	(Chu et al. 1985)
3	4-Butyl-piperazinyl	0.78	1.56	12.50	(Chu et al. 1985)
4	3-Methyl-piperazinyl	0.20	<0.05	0.78	(Chu & Fernandes 1991)
5	3,5-Dimethyl-piperazinyl	0.39	<0.05	1.56	(Chu & Fernandes 1991)
6	3-Fluoromethyl-piperazinyl	0.25	0.25	2.00	(Domagala et al. 1988)
7	3-Oxo-piperazinyl	0.20	0.20	1.56	(Chu et al. 1985)
8	4-Amino-piperazinyl	0.39	0.20	0.78	(Chu & Fernandes 1991)
9	Homopiperazinyl	0.79	0.20	1.56	(Chu et al. 1985)
10	Morpholinyl	0.10	0.39	3.10	(Chu et al. 1985)
11	Thiomorpholinyl	0.05	0.78	3.10	(Chu et al. 1985)
12	Piperidinyl	0.20	1.56	6.20	(Chu et al. 1985)
13	4-Hydroxy-piperidinyl	0.10	0.39	6.20	(Chu et al. 1985)
14	4-Dimethylamino-piperidinyl	0.39	0.73	3.10	(Chu et al. 1985)
15	Pyrrolidinyl	0.10	0.78	1.56	(Chu et al. 1985)
16	3-Hydroxy-pyrrolidinyl	0.10	0.20	3.10	(Chu et al. 1985)
17	3-Amino-pyrrolidinyl	0.10	<0.05	0.78	(Chu & Fernandes 1991)
18	3-Methylamino-pyrrolidinyl	0.20	<0.05	1.56	(Chu & Fernandes 1991)
19	3-Dimethylamino-pyrrolidinyl	0.78	<0.05	6.25	(Chu & Fernandes 1991)
20	3-Methyl-3-amino-pyrrolidinyl	0.05	0.02	0.78	(Rosen et al. 1988)
21	Trans (3-amino-2-methyl) -azetidinyl	0.25	0.12	1.00	(Domagala et al. 1988)
22	3-Amino-3-methyl-azetidinyl	0.12	0.12	2.00	(Domagala et al. 1988)

6-Fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acids substituted in position 7 with bioisosteric non-basic groups.

Table 8. The "in vitro" antibacterial activity of 1-(*p*-fluorophenyl)-7-substituted-quinolones.

4-(1,2,3,6-tetrahydropyridinyl) (compound 2), while the 3-amino-pyrrolidinyl moiety can be substituted with 3-amino-cyclopentanyl (compound 3). For the compounds 1 and 2, the nature of isosteric atom that replaces the nitrogen atom in position 1 of the piperazinyl moiety influences the antimicrobial activity "in vitro" as follows: while this sp^2 hybridized atom (through which connects the quinolone nucleus) leads to compounds (2) with activity comparable to ciprofloxacin, the presence of carbon sp^3 leads to decreased activity "in vitro" of about 4–12 times. Replacement of the 3-amino-1-pyrrolidinyl (compound B) with 3-amino-1-

cyclopentanyl (compound 3) results in a decrease in antimicrobial activity. In general, the piperazinyl moiety in position 7 has a favorable influence at the "in vitro" activity, but 7-substituted piperazinyl quinolones do not have a good availability.
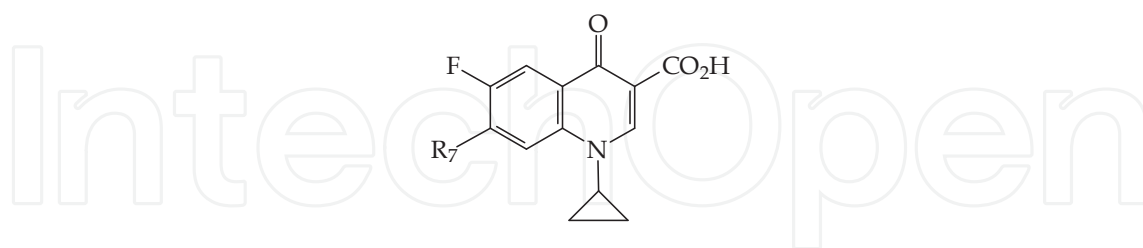
Figure 16. Cyclopropyl-quinolones substituted in position 7 with bioisosteric non-basic groups.

Compound	R_7	Minimum inhibitory concentration (µg / ml)			Reference
		S. aureus	*E. coli*	*P. aeruginosa*	
A	HN◯N—	0.13	0.03	0.13	(Bouzard et al. 1989)
1	HN◯—	0.80	0.20	1.60	(Laborde et al. 1993)
2	HN◯—	0.10	0.025	0.80	(Laborde et al. 1993)
B	H_2N◯N—	0.025	0.025	0.20	(Laborde et al. 1993)
3	H_2N◯N—	0.10	0.10	0.80	(Laborde et al. 1993)

Table 9. The "in vitro" antibacterial activity of 1-cyclopropyl-quinolones substituted in position 7 with bioisosteric non-basic groups.

2.8. Position 8

The most famous modifications of the 8 position of the quinolone are X = CH or N (naphthyridines). However, compact and lipophilic groups: X = CR_8, where R_8 = fluorine [16, 17, 25, 54], trifluoromethyl [52] and methoxy [22, 55] have gained ground due to the positive influence they have on the antibacterial activity.
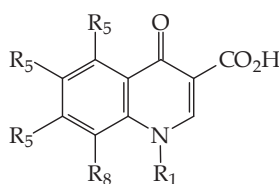
R₈ = F, Cl, OCH₃ > H, CF₃ > methyl, vinyl, propargyl

Figure 17. Influence of the nature of the substituent at 8 position on the antimicrobial activity.

In general, the introduction of a fluorine atom in 8 position leads to increased antibacterial activity against gram negative microorganisms, while introducing the methoxy group increase the activity only against the gram positive bacteria.
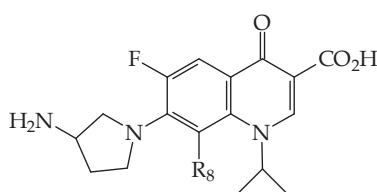
Figure 18. Cyclopropyl-8-substituted quinolones.

R₈	Minimum inhibitory concentration ($\mu G / ML$) geometric mean	
	Gram (-)organisms	Gram (+)organisms
H	0.09	0.14
F	0.04	0.03
OMe	0.07	0.03
Cl	0.03	0.04
NO₂	0.46	0.92
NH₂	0.53	0.06
CF₃	0.20	0.20
SMe	0.20	0.06

Table 10. The "in vitro" antibacterial activity 8 substituted quinolones.

The compounds containing a chlorine atom in 8 position [30, 25, 17] are very active, as well as analogs containing fluorine or methoxy moiety [22, 55]. The introduction of bulky substituents, e.g., ethyl, reduces the antibacterial activity against gram negative (if a comparison with 8-methoxy-quinolones is made). Quinolones having a halogen atom, fluorine or chlorine in 8

position, show the phototoxicity; the introduction of trifluoromethyl group [52] leads to reduced cytotoxicity. Alkylation of carbon in position 8 is tolerated, especially if the substituent is lipophilic and has a small volume. Thus the introduction of the methyl group [4, 5] leads to compounds with modest antibacterial activity. Instead, amino, nitro, hydroxy substituents lead to loss of antibacterial activity (Dax 1997).

3. New compounds: synthesis, structure and antimicrobial activity

3.1. Structure of the new compounds

A series of 4-oxo-1,4-dihydro-quinoline-3-carboxylic acids was synthesized. (Figure 19) (Table 11). Their structure has been determined and confirmed by the following physicochemical methods: elemental analysis, IR spectral analysis, H-NMR, C-NMR, UV, thin layer chromatography.
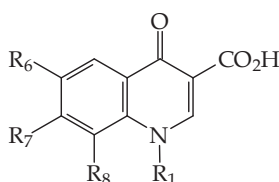
Figure 19. Structure of 4-oxo-1,4-dihydro-quinoline-3-carboxylic acids.

Quinolones	R_1	R_6	R_7	R_8	m.p. (°C)	Reference
FPQ 24 $C_{18}H_{21}FN_2O_3$	Ethyl	F	3-Methyl-piperidin-1-yl	H	189.4	Pintilie et al. (2009b)
FPQ 30 $C_{18}H_{20}ClFN_2O_3$	Ethyl	F	3-Methyl-piperidin-1-yl	Cl	163-165.3	Pintilie et al. (2014a)
FPQ 32 $C_{17}H_{19}FN_2O_3$	Ethyl	F	Piperidin-1-yl	H	202.4-204.4	Pintilie & Nita (2011a)
FPQ 33 $C_{17}H_{18}ClFN_2O_3$	Ethyl	F	Piperidin-1-yl	Cl	186-191.2	Pintilie & Nita (2011a)
Q 83 $C_{18}H_{21}FN_2O_3$	Ethyl	F	4-Methyl-piperidin-1-yl	H	235-237	Pintilie et al. (2003b)
Q 85 $C_{18}H_{20}ClFN_2O_3$	Ethyl	F	4-Methyl-piperidin-1-yl	Cl	201-202.5	Pintilie et al. (2003b)
FPQ 35 $C_{16}H_{17}FN_2O_3$	Ethyl	F	Pyrrolidin-1-yl	H	336.6-337.9	Pintilie & Nita (2011a)
FPQ 36	Ethyl	F	Pyrrolidin-1-yl	Cl	214.5-217.8	Pintilie & Nita (2011a)

Quinolones	R_1	R_6	R_7	R_8	m.p. (°C)	Reference
$C_{16}H_{16}ClFN_2O_3$						
FPQ 25 $C_{16}H_{17}FN_2O_4$	Ethyl	F	Morpholin-1-yl	H	257.4-258.7	Pintilie et al. (2009b)
FPQ 28 $C_{16}H_{16}ClFN_2O_4$	Ethyl	F	Morpholin-1-yl	Cl	244.6-244	Pintilie et al. (2014a)
NF $C_{16}H_{18}FN_3O_3$	Ethyl	F	Piperazin-1-yl	H	218-220	Pintilie et al. (2014b)
AcNF $C_{18}H_{20}FN_3O_4$	Ethyl	F	4-Acetyl-piperazin-1-yl	H	297.8-299.9	Pintilie et al. (2014b)
AcFPQ 50 $C_{18}H_{19}ClFN_3O_4$	Ethyl	F	4-Acetyl-piperazin-1-yl	Cl	255.7-258.2	Pintilie et al. (2014b)
FPQ 50 $C_{16}H_{17}ClFN_3O_3$	Ethyl	F	Piprazin-1-yl	Cl	227-230	Pintilie et al. (2014b)
PF $C_{17}H_{20}FN_3O_3$	Ethyl	F	4-Methyl-piperazin-1-yl	H	269.2-272.8	Pintilie et al. (2014b)
FPQ 51 $C_{17}H_{19}ClFN_3O_3$	Ethyl	F	4-Methyl-piperazin-1-yl	Cl	219.6-221	Pintilie et al. (2014b)
FPQ 27 $C_{17}H_{20}FN_2O_3$	Ethyl	F	3-Methyl-piperazin-1-yl	H	177.6-180.6	Pintilie et al. (2014a)
AcFPQ 27 $C_{19}H_{22}FN_3O_4$	Ethyl	F	4-Acetyl-3-methyl-piperazin-1-yl	H	247.5-248.9	Pintilie & Nita (2011a)
AcFPQ29 $C_{19}H_{21}ClFN_3O_4$	Ethyl	F	4-Acetyl-3-methyl-piperazin-1-yl	Cl	262.7-264.8	Pintilie & Nita (2011a)
FPQ29 . HCl $C_{17}H_{29}ClFN_2O_3$ .HCl	Ethyl	F	3-Methyl-piperazin-1-yl	Cl	280-283	Pintilie & Nita (2011a)
6ClPQ 24 $C_{18}H_{21}ClN_2O_3$	Ethyl	Cl	3-Methyl-piperidin-1-yl	H	216,4-218,4	Pintilie et al. (2009b)
6ClPQ 30 $C_{18}H_{20}Cl_2N_2O_3$	Ethyl	Cl	3-Methyl-piperidin-1-yl	Cl	190.3-192.2	Pintilie & Nita (2011b)
6ClPQ 32 $C_{17}H_{19}ClN_2O_3$	Ethyl	Cl	Piperidin-1-yl	H	234.6-236.4	Pintilie & Nita (2011b)
6ClPQ 33 $C_{17}H_{18}Cl_2N_2O_3$	Ethyl	Cl	Piperidin-1-yl	Cl	214.6-216.3	Pintilie & Nita (2011b)
PQ 80 $C_{18}H_{21}ClN_2O_3$	Ethyl	Cl	4-Methyl-piperidin-1-yl	H	262.5-264.4	Pintilie & Nita (2011b)
PQ87 $C_{18}H_{20}Cl_2N_2O_3$	Ethyl	Cl	4-Methyl-piperidin-1-yl	Cl	152.3-154.9	Pintilie & Nita (2011b)
6ClPQ 35 $C_{17}H_{18}Cl_2N_2O_3$	Ethyl	Cl	Pyrrolidin-1-yl	H	312.3-315.5	Pintilie & Nita (2011b)

Quinolones	R_1	R_6	R_7	R_8	m.p. (°C)	Reference
6ClPQ 36 $C_{17}H_{18}Cl_2N_2O_3$	Ethyl	Cl	Pyrrolidin-1-yl	Cl	172.4-176.1	Pintilie & Nita (2011b)
6ClPQ 25 $C_{16}H_{17}ClN_2O_4$	Ethyl	Cl	Morpholin-1-yl	H	267,1-269,2	Pintilie et al. (2009b)
6ClPQ 28 $C_{16}H_{16}Cl_2N_2O_4$	Ethyl	Cl	Morpholin-1-yl	Cl	213.9-216.7	Pintilie & Nita (2011b)
NClX $C_{16}H_{18}ClN_3O_3$	Ethyl	Cl	Piperazin-1-yl	H	226.8-228.5	Pintilie et al. (2014b)
AcNClX $C_{18}H_{20}ClN_3O_4$	Ethyl	Cl	4-Acetyl-piperazin-1-yl	H	306-310	Pintilie et al. (2014b)
Ac6ClPQ 50 $C_{18}H_{19}Cl_2N_3O_4$	Ethyl	Cl	4-Acetyl-piperazin-1-yl	Cl	260.1-263.7	Pintilie et al. (2014b)
6ClPQ 50 $C_{16}H_{17}Cl_2N_3O_3$	Ethyl	Cl	Piperazin-1-yl	Cl	228.2-230.4	Pintilie et al. (2014b)
PClX $C_{17}H_{20}ClN_3O_3$	Ethyl	Cl	4-Methyl-piperazin-1-yl	H	253.7-258.2	Pintilie et al. (2014b)
6ClPQ 51 $C_{17}H_{19}Cl_2N_3O_3$	Ethyl	Cl	4-Methyl-piperazin-1-yl	Cl	223.4-226	Pintilie et al. (2014b)
6ClPQ 27 $C_{17}H_{20}ClN_3O_3$	Ethyl	Cl	3-Methyl-piperazin-1-yl	H	170.5-171.4	Pintilie et al. (2009b)
Ac6ClPQ 27 $C_{19}H_{22}ClN_3O_4$	Ethyl	Cl	4-Acetyl-3-methyl-piperazin-1-yl	H	275-276	Pintilie et al. (2014 b)
Ac6ClPQ 29 $C_{19}H_{21}Cl_2N_3O_4$	Ethyl	Cl	4-Acetyl-3-methyl-piperazin-1-yl	Cl	210.3-211.7	Pintilie et al. (2014b)
6ClPQ 29 $C_{17}H_{19}Cl_2N_2O_3$	Ethyl	Cl	3-Methyl-piperazin-1-yl	Cl	278.5-282	Pintilie et al. (2014b)

Table 11. Oxo-1,4-dihydro-quinoline-3-carboxylic acids synthesized.

3.2. Synthesis pathway

The synthesis of the novel quinolones followed a Gould–Jacobs cyclization process (Scheme 1). Appropriate unsubstituted aniline (1) is reacted with diethylethoxy methylene malonate (EMME) to produce the resultant anilinomethylenemalonate. A subsequent thermal process induces Gould–Jacobs cyclization to afford the corresponding 4-hidroxy-quinoline-3-carboxylate ester (2). The following operation is the alkylation of the quinolone, which is usually accomplished by reaction with a suitable alkyl halide or dialkyl sulphates to produce the qinolone-3-carboxylate ester (3). The final manipulation is acid or basic hydrolysis to cleave the ester generating the biologically active free carboxylic acid (4). The biologically active free carboxylic acid (4) was also obtained from the corresponding 4-hidroxy-quinoline-3-carboxylate ester (2) by alkylation with dialkyl sulphates in presence of alkali, for example, the reaction

it can conveniently be carried out in aqueous 40% sodium hydroxide solution. The displacement of 7-chloro group with a heterocyclic yielded compounds (5). 8-Chloro-quinoline-3-carboxylic acid (8) was synthesized from 8-unsubstituted quinoline-3-carboxylic acid (5) by chlorination with sulfuryl chloride (when R_7 = 4-methyl-piperazine). When R_7 = 3-methyl-piperazine or piperazine, it is necessary to protect the nitrogen atom from piperazine group. After chlorination and hydrolysis, the final compound (8) is obtained (R_7 = 3-methyl-piperazine, or piperazine).
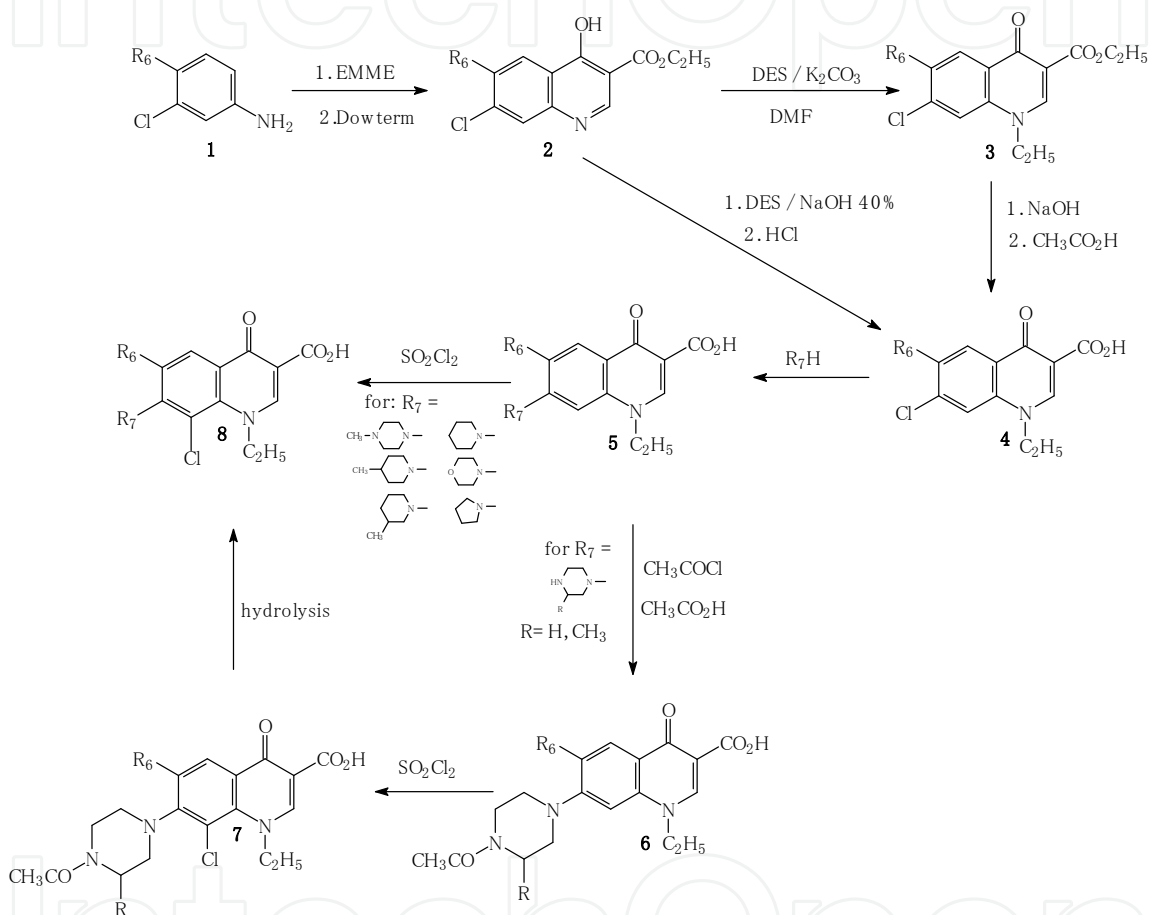

Figure 20. Synthesis of the new quinolones.

3.3. Antibacterial activity of the new compounds

The new compounds were evaluated for "in vitro" activity by determining minimum inhibitory concentration against bacteria *E. coli, S. aureus*, and *P. aeruginosa*, by agar dilution method (Buiuc 1998) (NCCLS 2003) (Table 12). After analyzing chemical structure–biological activity relationships, it was observed that the presence of chlorine in 8 position of the quinolones core leads to increased antimicrobial activity for the compounds having piperidinyl, morpholinyl, and pyrrolidinyl moiety in 7-position. For 7-piperazinyl quinolones, the chlorine atom from 8-position leads to decreased activity against all the tested strains.

Quinolone	Minimum inhibitory concentration µg/ml			References
	E. coli (a)	*S. aureus* (b)	*P. aeruginosa* (c)	
FPQ 24	2.00	0.50	32.00	Pintilie et al. 2009b
FPQ 30	0.32	0.125	1.28	Pintilie & Nita 2011a
FPQ 32	1.00	8.00	8.00	Pintilie & Nita 2011a
FPQ 33	0.32	0.32	1.28	Pintilie & Nita 2011a
Q 83	3,12	1,56	6,25	Pintilie et al. 2003b
Q 85	3,12	0,39	6,25	Pintilie et al. 2003b
FPQ 35	31.25	1.953	"/>125	Pintilie & Nita 2011a
FPQ 36	15.625	0.244	15.625	Pintilie & Nita 2011a
FPQ 28	0,125	0,06	8,00	Pintilie et al. (2009b)
NF	<0.08	0.32	0.32	Pintilie et al. 2014 b
FPQ 50	2.00	4.00	16.00	Pintilie et al. 2014 b
PF	<0.08	1.28	1.28	Pintilie et al. 2014 b
FPQ 51	2.00	4.00	32.00	Pintilie et al. 2014 b
FPQ 27	0.125	1.00	1.00	Pintilie & Nita 2011a
FPQ 29	0.30	1.21	4.83	Pintilie & Nita 2011a
6ClPQ 24	8,00	2,00	"/>128	Pintilie et al. (2009b)
6ClPQ 30	8.00	2.56	2.56	Pintilie & Nita 2011b
6ClPQ 33	2.56	2.56	2.56	Pintilie & Nita 2011b
6ClPQ 36	2.56	2.56	2.56	Pintilie & Nita 2011b
6ClPQ 28	1.28	1.28	64	Pintilie & Nita 2011b
NClX	0.32	1.28	5.12	Pintilie et al. 2014 b
6ClPQ 50	2.00	8.00	32.00	Pintilie et al. 2014 b
PClX	0.32	1.28	5.12	Pintilie et al. 2014 b
6ClPQ 51	3.906	15.625	62.5	Pintilie et al. 2014 b

a. *E. coli* ATCC 8739, b. *S. aureus* ATCC 6538, c. *P. aeruginosa* ATCC 9027

Table 12. "In vitro" antibacterial activity of the new quinolones.

3.4. Antibacterial activity of the new compounds against methicillin-resistant *S. aureus*

Some of the new quinolones, which showed a good activity, have been tested against 30 strains of methicillin-resistant *S. aureus* isolated in the Microbiology Laboratory of INBI Prof. "Dr. Matei Bals" during 2012. The minimum inhibitory concentration (MIC) of the isolates has been determined by agar plate Mueller Hinton (bioMerieux) dilution method using the reference strain *S. aureus* ATCC 29213. The 30 strains of isolated have been also tested for susceptibility to ciprofloxacin, levofloxacin, and imipenem by Etest method.
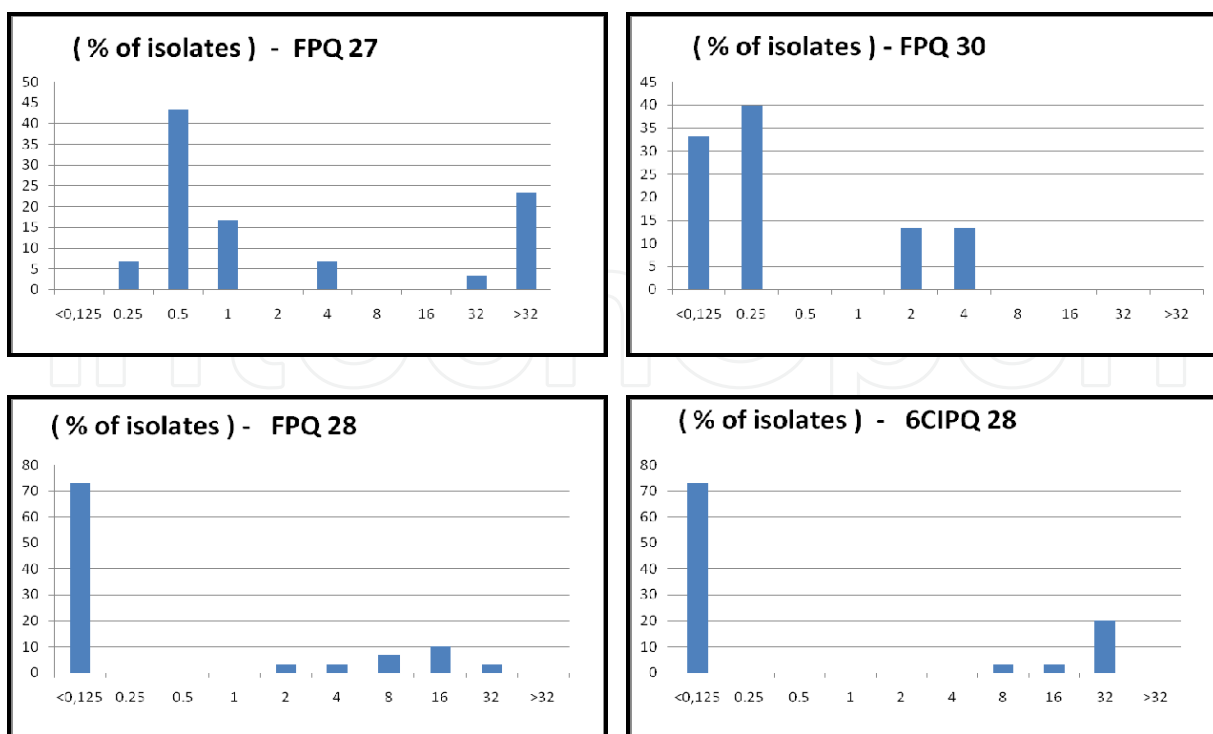
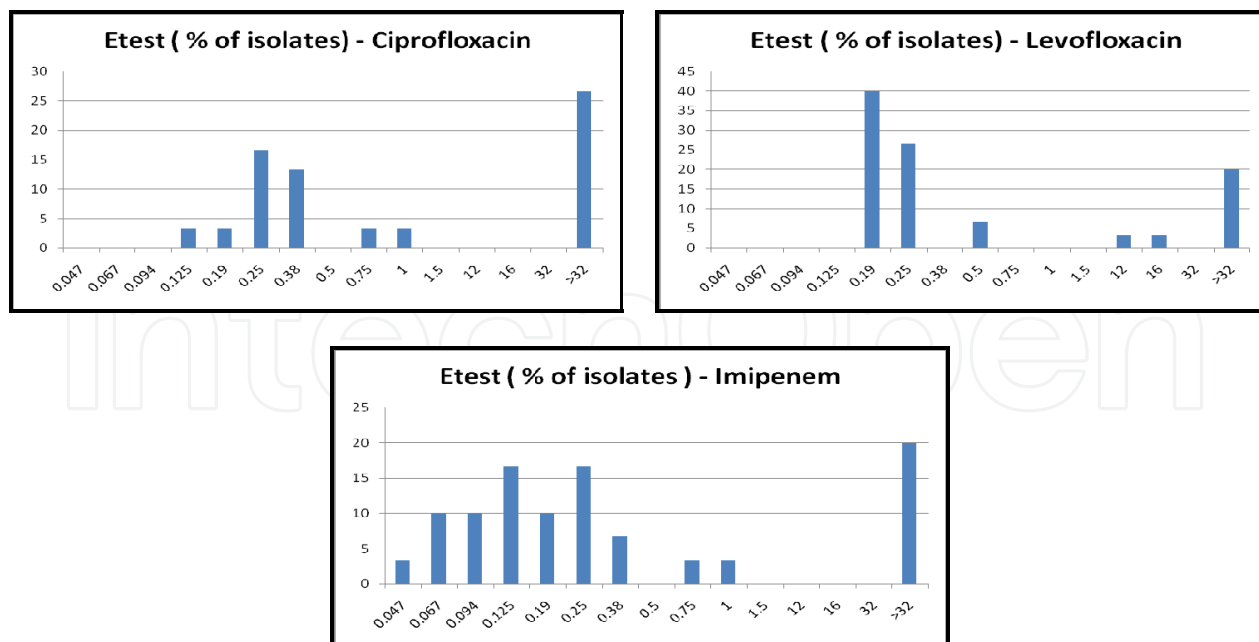**Figure 21.** MIC histograms of 4 quinolones against 30 strains of methicillin-resistant *S. aureus*.


Figure 22. MIC histograms of ciprofloxacin, levofloxacin, imipenem against 30 strains of methicillin-resistant *S. aureus*, determined by Etest method.

Base on the "in vitro" studies, the quinolone FPQ-30 appears to be a promising compound; all strains isolates were inhibited at a concentration of 8 μg/ml (Figure 21).

4. Conclusion

In conclusion, we have synthesized new quinolone compounds and we have investigated their activity against multidrug-resistant gram positive microorganisms. Of the four compounds, FPQ-30 (Figure 23, 24) showed the best activity; all strains isolates were inhibited at a concentration of 8 μg/ml. The results of the present study indicate the quinolone FPQ-30 appears to be a promising compound.

Its structure has been determined and confirmed by the following physicochemical methods: elemental analysis, IR spectral analysis, H-NMR, C-NMR, UV (Figure 25) and thin layer chromatography.
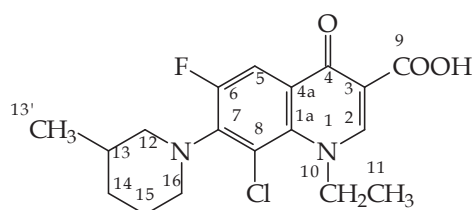
^{1}H-NMR(dmso-d6, δ ppm, J Hz, T = 333K): 8.88(s, 1H, H-2); 7.93(d, 1H, H-5, ^3J(F-H^5) = 13.4 Hz); 4.81(q, 2H, H-10, 7.1); 3.28(m, 2H, H-16, H-12); 3.11(dd, 1H, H-12, 10.7, 12.1); 2.81(td, 1H, H-16, 12.1, 2.9); 1.58 ÷ 1.86(m, H, H-13, 2H-14, 1H-15); 1.39(t, 3H, H-11, 7.1); 1.13(qd, 1H, H-15, 10.8, 4.1); 0.88(d, 3H, H-13, 6.4).

^{13}C-NMR(dmso-d6, δ ppm): 175.89(d, C-4, ^4J(F-C^4) = 2.8 Hz); 167.30(C-9); 155.83(d, C-6, J(F-C^6) = 251.1 Hz); 152.45(C-2); 144.37(d, C-7, ^2J(F-C^7) = 14.3 Hz); 136.46(C-1a); 122.95(d, C-4a, ^3J(F-C^{4a}) = 7.1 Hz); 118.93(C-8); 110.79(d, C-5, ^2J(F-C^5) = 23.4 Hz); 107.67(C-3); 58.70 (d, C-12, ^4J(F-C^{12}) = 4.6 Hz); 53.06(C-10); 51.57(d, C-16, ^4J(F-C^{16}) = 4.6 Hz); 32.05(C-15); 31.17(C-13); 25.39(C-14); 18.82(C-13);15.73(C-11).

FT-IR(solid in ATR, ν/cm): 3059m; 2947w; 2925m; 2867w; 2845m; 1719vs; 1616s; 1558s; 1531m; 1489m; 1437vs; 1381m; 1319m; 1294m; 1250m; 1229m; 1208m; 1189m; 1125w; 1101m; 1081m; 1040m; 996w; 968w; 926m; 887m; 857w; 838w; 8.05m; 741w.

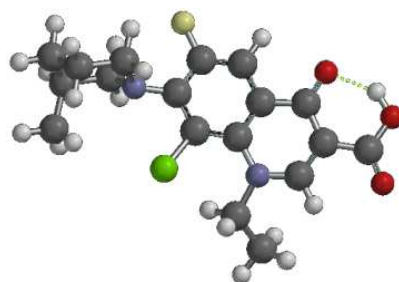**Figure 23.** Ethyl-6-fluoro-7-(4-methyl-piperidinyl)-8-chloro- Optimized molecular structure of FPQ 30

Figure 24. dihydro-4-oxo-quinoline-3-carboxylic acid (FPQ 30) with Spartan 14 Software
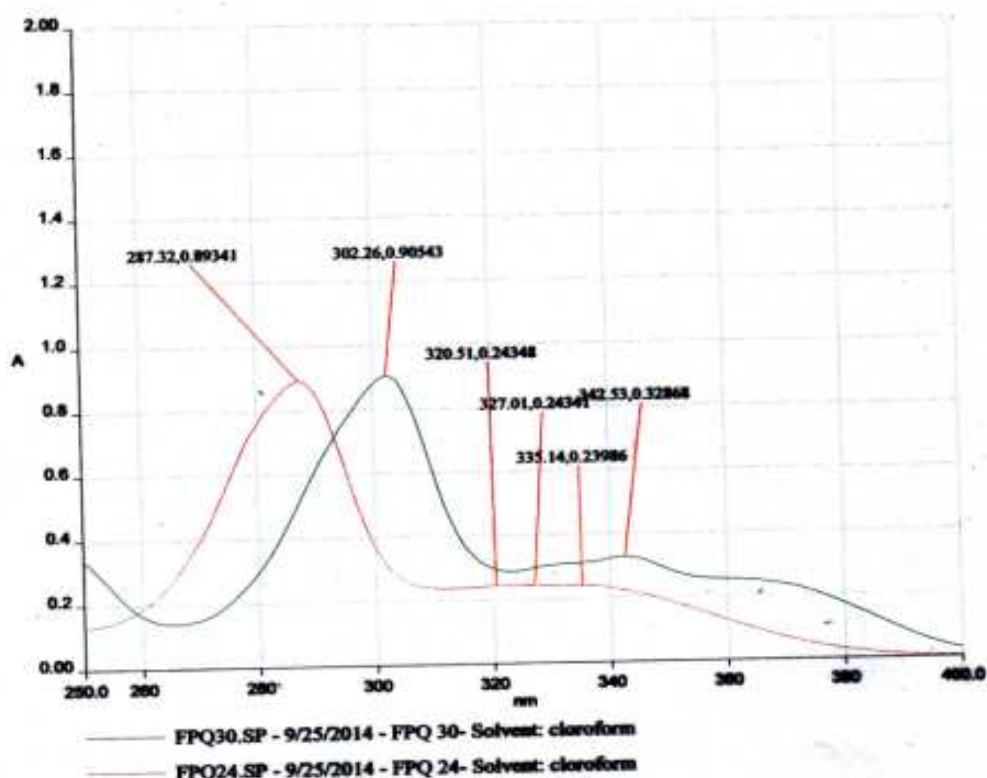
Figure 25. UV absorption spectrum of FPQ-24 and FPQ-30

UV absorption spectra study was carried out using solutions with oncentration of 10 mg/ml in chloroform. Interpretation of the UV absorption spectrum has been made in comparison with that of the quinolone compound: 1-ethyl-6-fluoro-7-(3-methyl-piperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (FPQ 24), namely:

- The presence of the quinolone nucleus determines in chloroform the appearance of electronic transitions in the field of 260–310 nm and 320–350 nm.

- The introduction of chlorine atom in 8 position produces a batocroma displacement of the entire spectrum.

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References

[1] Bouzard D, DiCesare P, Essiz M, Jacquet JP, Remuzon P, Weber A, Oki T, Masuyoshi M. Fluoronaphthyridines and quinolones as antibacterial agents. 1. Synthesis and structure-activity relationship of new 1-substituted derivatives. J Med Chem 1989;32(3):537–42, ISSN-0022-2623.

[2] Bush AR, Domagala JM, Laborde E, Sesnie JC. Synthesis and antimicrobial evaluation of a series of 7-[3-amino (or aminomethyl)-4-aryl (or cyclopropyl)-1-pyrrolidinyl]-4-quinolone and -1,8-naphthyridone-3-carboxylic acids. J Med Chem 1993;36(26):4139–59, ISSN-0022-2623.

[3] Cecchetti V, Clementi S, Cruciani G, Fravolini A, Pagella PG, Savino A, Tabarini O. 6-Aminoquinolones: a new class of quinolone antibacterials? J Med Chem 1995;38(6): 973–82, ISSN-0022-2623.

[4] Cechetti V, Fravolini A, Lorenzini MC, Tabarrini O, Terni P, Xin T. Studies on 6-Aminoquinolones: synthesis and antibacterial evaluation of 6-Amino-8-methylquinolones. J Med Chem 1996a;39(2):436–45, ISSN-0022-2623.

[5] Cechetti V, Fravolini A., Palumbo M, Sissi C, Tabarrini O, Terni P, Xin, T. Potent 6-desfluoro-8-methylquinolones as new lead compounds in antibacterial chemotherapy. J Med Chem 1996b:39(25):4952–7, ISSN-0022-2623.

[6] Cechetti V, Filipponi E, Fravolini A, Tabarrini O, Bonelli D, Clementi M, Cruciani G, Clementi S. Chemometric methodologies in a quantitative structure−activity relationship study: the antibacterial activity of 6-aminoquinolones. J Med Chem 1997;40(11):1698–706, ISSN-0022-2623.

[7] Chu DTW, Fernandes P, Claiborne AK, Pihuleac E, Norden CW, Maleczka JRE, Pernet AG. Synthesis and structure-activity relationships of novel arylfluoroquinolone antibacterial agents. J Med Chem 1985;28(12):1558–64, ISSN-0022-2623.

[8] Chu DTW, Fernandes PB, Maleczka Jr RE, Nordeen CW, Pernet AG. Synthesis and structure-activity relationship of 1-aryl-6,8-difluoroquinolone antibacterial agents. J Med Chem 1987;30(3):504–9, ISSN-0022-2623.

[9] Chu DTW, Fernandes P. Recent developments in the field of quinolone antibacterial agents. In: Advances in Drug Research, 1991, Vol. 21, Bernard Testa, pp.39–144, Academic Press, ISBN 0-12-013321-0, London; San Diego; New York.

[10] Cooper CS, Klock PL, Chu DTW, Fernandes PB. The synthesis and antibacterial activities of quinolones containing five- and six-membered heterocyclic substituents at the 7-position. J Med Chem, 1990;33(4):1246–52, ISSN-0022-2623.

[11] Cooper CS, Klock PL, Chu DTW, Hardy DJ, Swanson RN, Plattner JJ Preparation and in vitro and in vivo evaluation of quinolones with selective activity against Gram-positive organisms. J Med Chem 1992;35(8):1392–8, ISSN-0022-2623.

[12]   Culbertson TP, Domagala JM, Peterson P, Bongers S, Nichols JB. New 7-substituted quinolone antibacterial agents. The synthesis of 1-ethyl-1,4-dihydro-4-oxo-7-(2-thiazolyl and 4-thiazolyl)-3-quinolinecarboxylic acids. J Heterocyclic Chem 1987;24(6): 1509–20, ISSN-0022-152X.

[13]   Culbertson TP, Sanchez JP, Gambino L, Sesnie JA. Quinolone antibacterial agents substituted at the 7-position with spiroamines. Synthesis and structure-activity relationships. J Med Chem 1990;33(8)2270–5, ISSN-0022-2623.

[14]   Di Cesare P, Bousard D, Essiz M, Jacquet JP, Ledoussal B, Kiechel JR, Remuzon P, Kessler RE, Fung-Tomc J, Desiderio J. Fluoronaphthyridines and -quinolones as antibacterial agents. 5. Synthesis and antimicrobial activity of chiral 1-tert-butyl-6-fluoro-7-substituted-naphthyridones. J Med Chem 1992;35(22): 4205–13, ISSN-0022-2623.

[15]   Domagala JM, Heifetz CL, Mich TF, Nichols JB. 1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid. New quinolone antibacterial with potent gram-positive activity. J Med Chem 1986;29(4): 445–8, ISSN-0022-2623.

[16]   Domagala JM, Hagen SE, Heifetz CL, Hutt MO, Mich TF, Sanchez JP, Trehan AK. 7-Substituted 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids: synthesis and biological activity of a new class of quinolone antibacterials. J Med Chem 1988;31(3):503–6, ISSN-0022-2623.

[17]   Domagala JM, Bridges AJ, Culbertson TP, Gambino L, Hagen SE, Karrick G, Porter K, Sanchez JP, Sesnie J.A. Synthesis and biological activity of 5-amino- and 5-hydroxyquinolones, and the overwhelming influence of the remote N1-substituent in determining the structure-activity relationship. J Med Chem 1991;34(3):1142–54, ISSN-0022-2623.

[18]   Domagala JM, Hagen SE, Joannides T, Kiely JS, Laborde E, Schroeder MC, Sesnie JA, Shapiro MA, Suto MJ, Vanderroest S. Quinolone antibacterials containing the new 7-[3-(1-aminoethyl)-1-pyrrolidinyl] side chain: the effects of the 1-aminoethyl moiety and its stereochemical configurations on potency and in vivo efficacy. J Med Chem 1993;36(7):871–82, ISSN-0022-2623.

[19]   Frigola J, Peres J, Corbera J, Vano D, Merce R, Antoni T, Mas J, Valenti E. 7-Azetidinylquinolones as antibacterial agents. Synthesis and structure-activity relationships. J Med Chem 1993;36(7):801–10, ISSN-0022-2623.

[20]   Frigola J, Torrens A, Castrillo JM, Vano D, Berrocal JM, Calvet C, Salgado L, Redondo J. 7-Azetidinylquinolones as antibacterial agents. 2. Synthesis and biological activity of 7-(2,3-disubstituted-1-azetidinyl)-4-oxoquinoline- and -1,8-naphthyridine-3-carboxylic acids. Properties and structure-activity relationships of quinolones with an azetidine moiety. J Med Chem 1994;37(24):4195–210, ISSN-0022-2623.

[21]   Frigola J, Vano D, Torrens A, Gomez-Gomar A, Ortega E, Garcia-Granda S. 7-Azetidinylquinolones as antibacterial agents. 3. Synthesis, properties and structure-

activity relationships of the stereoisomers containing a 7-(3-amino-2-methyl-1-azetidinyl) moiety. J Med Chem 1995;38(7):1203–15, ISSN-0022-2623.

[22] Fukui H, Shibata T, Naito T, Nakano J, Maejima T, Senda H, Iwatani W, Tatsumi Y, Suda M, Arika T. Synthesis and antibacterial activity of novel 7-(3-substituted-3 or 4-trifluoromethyl-1-pyrrolidinyl)-8-methoxyfluoroquinolones. Bioorg Med Chem Lett 1988;8(20):2833–8, ISSN: 0960-894X.

[23] Hagen SE, Domagala JM, Heifetz CL, Sanchez JP, Solomon M. New quinolone antibacterial agents. Synthesis and biological activity of 7-(3,3- or 3,4-disubstituted-1-pyrrolidinyl)quinoline-3-carboxylic acids. J Med Chem 1990a;33(2):849–54, ISSN-0022-2623.

[24] Hagen ES, Domagala MJ. Synthesis of 5-methyl-4-oxo-quinolinecarboxylic acids. J Heterocyclic Chem 1990b;27(6):1609–16, ISSN-0022-152X.

[25] Hagen ES, Domagala JM, Heifetz CL, Johnson J. Synthesis and biological activity of 5-alkyl-1,7,8-trisubstituted-6-fluoroquinoline-3-carboxylic acids. J Med Chem 1991;3(3):1155–61, ISSN-0022-2623.

[26] Hagen SE, Domagala JM, Gracheck SJ, Sesnie JA, Stier MA, Suto MJ. Synthesis and antibacterial activity of new quinolones containing a 7-[3-(1-amino-1-methylethyl)-1-pyrrolidinyl] moiety. Gram-positive agents with excellent oral activity and low side-effect potential. J Med Chem 1994;37(6):733–8, ISSN-0022-2623.

[27] Isao H, Attashi S.. Eur. Pat. Appl. EP 01 911 85, 1990.

[28] Itoh Y, Kato H. Ger. Offen DE 34 33 924 A1, 1984.

[29] Jose A, Fromtling RA, Bland JA, Pelak BA, Gilfillan EC. (Acyloxy)alkyl carbamate prodrugs of norfloxacin. J Med Chem 1991;34(1):78–81, ISSN-0022-2623.

[30] Kaziko A, Tsuyoshi K, Uemori S, Moriguchi A, Ikeda Y, Hirayama F, Yokoyama Y, Iwao E, Yakushiji T. Quinolone antimicrobial agents substituted with morpholines at the 7-position. Syntheses and structure-activity relationships. J Med Chem 1993;36(10):1356–63, ISSN-0022-2623.

[31] Kondo H, Sakamato F, Kawakami K, Tsukamoto G. Studies on prodrugs. 7. Synthesis and antimicrobial activity of 3-formylquinolone derivatives. J Med Chem 1988;31(1):3344–52, ISSN-0022-2623.

[32] Koga H, Itoh A, Murayama S. Structure-activity relationships of antibacterial 6,7- and 7,8-disubstituted 1-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids. J Med Chem 1980;23(12):1358–1363, ISSN-0022-2623.

[33] Laborde E, Kiely JS, Culbertson TP, Lesheski LE. Quinolone antibacterials: synthesis and biological activity of carbon isosteres of the 1-piperazinyl and 3-amino-1-pyrrolidinyl side chains. J Med Chem 1993;36(14):1964–70, ISSN-0022-2623.

[34] Liu J, Guo H. Synthesis and antibacterial activity of 1-(substituted pyrrolyl)-7-(substituted amino)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids. J Med Chem 1992;35(19):3469–73, ISSN-0022-2623.

[35] Moran BD, Ziegler BC, Dunne TS, KucK NA, Lin YI. Synthesis of novel 5-fluoro analogs of norfloxacin and ciprofloxacin. J Med Chem 1989;32(12):1313–8, ISSN-0022-2623.

[36] Myamoto T, Matsumoto J, Chiba K, Egawa H, Shibamori K, Minamida A, Nishimura Y, Okada H, Kataoka M. Pyridonecarboxylic acids as antibacterial agents. Part 14. Synthesis and structure-activity relationships of 5-substituted 6,8-difluoroquinolones, including sparfloxacin, a new quinolone antibacterial agent with improved potency. J Med Chem 1990;33(6):1645–56, ISSN-0022-2623.

[37] Ohta M, Koga H. Three-dimensional structure-activity relationships and receptor mapping of N1-substituents of quinolone antibacterials. J Med Chem 1991;34(1):131–9, ISSN-0022-2623.

[38] Pesson M. Ger. Offen Patent DE 28 409 10, 1979.

[39] Petersen U, Grohe K. Ger. Offen Patent DE 32 485 06, 1984b.

[40] Pintilie L, Oniscu C, Voiculescu Gh, Draghici C, Caproiu MT, Alexandru N, Paraschiv I, Damian E, Dobrovolschi D, Diaconu L. Synthesis and antibacterial activity of some novel fluoroquinolones. Romanian Biotechnol Lett 2003b;8(2):1197–204. ISSN 1224-5984.

[41] Pintilie L, Negut C, Oniscu C, Caproiu MT, Nechifor M, Iancu L, Ghiciuc C, Ursu R. Synthesis and antibacterial activity of some novel quinolones. Romanian Biotechnol Lett 2009b;14(5):4756–67, ISSN 1224-5984.

[42] Pintilie L, Nita S. RO *Patent application* RO A/01346, 07.12.2011a.

[43] Pintilie L, Nita S. RO *Patent application* RO A/01345, 07.12.2011b.

[44] Pintilie L, Dorobat O, Caproiu MT, Maganu M. Quinolone derivatives with activity against multi-drug resistant gram-positive microorganisms. Revista de chimie 20141;65(10):1176–81, ISSN 0034-7752.

[45] Pintilie L, Nicu AI, Stefaniu A, Deaconu M, Caproiu MT. RO *Patent application ROA/*00803/28.10.2014b.

[46] Radl S, Zikan V. Synthesis of some 1-aryl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acids and their antibacterial activity. Collect. Czech Chem Commun 1989;54(8):2181–9, ISSN – 0010-0765.

[47] Remuzon P, Bouzard D, Di Cesare P, Essiz M, Jacquet JP, Kiechel JR, Ledoussal B, Kessler RE, Fung-Tomc J. Fluoronaphthyridines and -quinolones as antibacterial agents. 3. Synthesis and structure-activity relationships of new 1-(1,1-dimethyl-2-fluoroethyl), 1-[1-methyl-1-(fluoromethyl)-2-fluoroethyl], and 1-[1,1-(difluoromethyl)-2-fluoroethyl] substituted derivatives. J Med Chem 1991;34(1):29–37, ISSN-0022-2623.

[48] Renau TE, Sanchez JP, Gage JW, Dever JA, Shapiro MA, Gracheck SJ, Domagala JM. Structure–activity relationships of the quinolone antibacterials against mycobacteria: effect of structural changes at N-1 and C-7 J Med Chem 1996;39(3):799–835, ISSN-0022-2623.

[49] Reuman M, Daum SJ, Singh B, Wentland MP, Perni RB, Pennock P, Carabateas PM, Gruett MD, Saindane MT, Dorff PH, Coughlin SA, Sedlock DM, Rake JB, Lesher GY. Synthesis and antibacterial activity of some novel 1-substituted 1,4-dihydro-4-oxo-7-pyridinyl-3-quinolinecarboxylic acids. Potent antistaphylococcal agents. J Med Chem 1995;38(14):2531–40, ISSN-0022-2623.

[50] Rosen T, Chu DTW, Lico IM, Fernandes PB, Marsh K, Shen L, Cepa VG, Pernet AG. Design, synthesis, and properties of (4S)-7-(4-amino-2-substituted-pyrrolidin-1-yl)quinolone-3-carboxylic acids. J Med Chem 1988b;31(8):1598–611, ISSN-0022-2623.

[51] Sakamato F, Ikeda S, Kondo H, Tsukamoto G.. Studies on prodrugs. IV. Preparation and characterization of N-(5-substituted 2-oxo-1,3-dioxol-4-yl)methyl norfloxacin. Chem Pharm Bull1986;33(11):4870–7, ISSN- ISSN: 0009-2363.

[52] Sanchez JP, Bridges AJ, Bucsh R, Domagala JM, Gogliotti RD, Hagen SE, Heifetz CL, Joannides ET, Sesnie JC. New 8-(trifluoromethyl)-substituted quinolones. The benefits of the 8-fluoro group with reduced phototoxic risk. J Med Chem 1992a;35(2):361–7, ISSN-0022-2623.

[53] Sanchez JP, Domagala JM, Heifetz CL, Priebe SR, Sesnie JA, Trehan AK. Quinolone antibacterial agents. Synthesis and structure-activity relationships of a series of amino acid prodrugs of racemic and chiral 7-(3-amino-1-pyrrolidinyl)quinolones. Highly soluble quinolone prodrugs with in vivo pseudomonas activity. J Med Chem 1992b; 35(10):1764–73, ISSN-0022-2623.

[54] Sanchez JP. J Med Chem 1988;31(10):983–91, ISSN-0022-2623.

[55] Sanchez JP. J Med Chem 1995;38(10)4478–87, ISSN-0022-2623.

[56] Scriewer M, Petersen U, Grohe K. Eur. Pat. Appl EP 03 320 33, 1988a.

[57] Scriewer M, Petersen U, Grohe K. Ger. Offen Patent DE 3808 118, 1988b.

[58] Scott LD Quinolone antibacterial. In: Antibacterial Chemotherapeutic Agents, 1997, pp. 298-345, Blackie Academic & Professional, London, ISBN 10 0751402893.

[59] Segawa J, Kitano M, Kazuno K, Matsuoka M, Shirahase I, Ozaki M, Matsuda M, Tomii Y, Kise M. Studies on pyridonecarboxylic acids. 1. Synthesis and antibacterial evaluation of 7-substituted-6-halo-4-oxo-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acids. J Med Chem 1992;35(25)4727–38, ISSN-0022-2623.

[60] Shongo A, Imamura M, Kimura Y, Yoshida A, Hayakawa I. Fluorocyclopropyl quinolones. 1. Synthesis and structure-activity relationships of 1-(2-fluorocyclopropyl)-3-pyridonecarboxylic acid antibacterial agents. J Med Chem 1993;36(22)3444–8, ISSN-0022-2623.

[61] Suto MJ, Domagala JM, Roland GE, Mailloux GB, Cohen MA. Fluoroquinolones: relationships between structural variations, mammalian cell cytotoxicity and antimicrobial activity. J Med Chem 1992;35(25):4745–50, ISSN-0022-2623.

[62] Taguchi M, Kondo H, Inoue Y, Kawahata Y, Jinbo Y, Sakamoto F, Tsukamoto G. Synthesis and antibacterial activity of new tetracyclic quinolone antibacterials. J Med Chem 1992;35(1):94–9, ISSN-0022-2623.

[63] Toshio U, Takamatsu M, Inoue Y, Kawahata Y, Iuchi K, Tsukamoto G. Synthesis of antimicrobial agents. 1. Syntheses and antibacterial activities of 7-(azole substituted)quinolones. J Med Chem 1987;30(120)2163–9, ISSN-0022-2623.

[64] Youichi Kimura Y, Atarashi S, Kawakami K, Sato K, Hayakawa I. Fluorocyclopropyl)quinolones. 2. Synthesis and stereochemical structure-activity relationships of chiral 7-(7-amino-5-azaspiro[2.4]heptan-5-yl)-1-(2-fluoro cyclopropyl) quinolone antibacterial agents. J Med Chem 1994;37(20)3344–52, ISSN-0022-2623.

[65] Wentland MP, Bailey DM, Cornett JB, Dobson RA, Powless RG, Wagner RB. Novel amino-substituted 3-quinolinecarboxylic acid antibacterial agents: synthesis and structure-activity relationships. J Med Chem 1984;27(9)1103–1, ISSN-0022-2623.

[66] Wentland MP, Lesher GY, Reuman M, Gruett MD, Singh B, Aldous SC, Dorff PH, Rake JB, Coughlin SA. Mammalian topoisomerase II inhibitory activity of 1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic acid and related derivatives. J Med Chem 1993;36(19):2801–9, ISSN-0022-2623.

[67] Ziegler Jr CB, Bitha P, Kuck NA, Fenton TJ, Petersen PJ, Lin YI. Synthesis and structure-activity relationships of new 7-[3-(fluoromethyl) piperazinyl]- and -(fluorohomopiperazinyl)quinolone antibacterials. J Med Chem 1990;33(1):142–6, ISSN-0022-2623.

[68] Ziegler Jr GB, Nydia AK, Strohmeyer TW, Lin YL. Synthesis and In vitro biological activity of some 7-(5-aminomethyl-2-isoxazolidinyl)quinolone-3-carboxylic acids. J Heterocyclic Chem 1990;27(6):2077–9, ISSN-0022-152X.