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Fluoroquinolone derivatives in the treatment of mycobacterium tuberculosis infection

João Pais[1], Margarida Policarpo[2], David Pires[1], Bernard Testa[3], Elsa Anesa[1,2], and Luís Constantino[1,2,*]

[1] Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal;
[2] Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal;
[3] University of Lausanne, 1015 Lausanne, Switzerland

* Corresponding author: constant@ff.ul.pt

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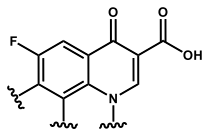
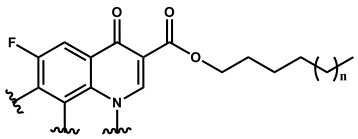
Fluoroquinolone derivatives in the treatment of mycobacterium tuberculosis infection

Ester Formation



Fluoroquinolones (FQ)



Fluoroquinolone esters (FQE)

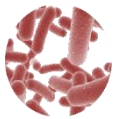
Antitubercular activity

TB

MIC 2,5 µg/ml

Antimicrobial activity

Gram⁺ Susceptible

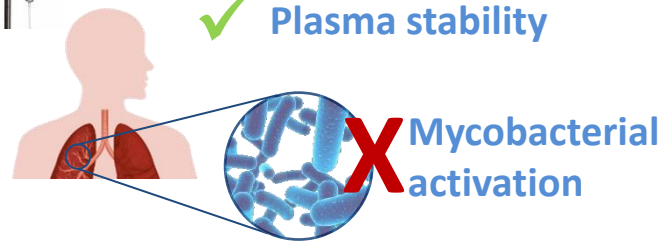


Gram⁺ **Resistant**

FQ	>4 µg/ml	128 µg/ml
FQE	**8 µg/ml**	**8 µg/ml**

✓ **Chemical stability**

✓ **Plasma stability**



X **Mycobacterial activation**

- Not a prodrug – a new drug
- Broad antibacterial action
- **Active against FQ-resistant bacteria**

Abstract:

Tuberculosis (TB) is currently one of the leading causes of death due to infective agents and the growing rate of multidrug-resistant tuberculosis (MDR TB) cases, is an emergent public health threat. Fluoroquinolones are commonly used in the treatment of tuberculosis for drug-sensitive patients who are intolerant to first-line antitubercular agents, as well as in the case of MDR TB. Unfortunately, these drugs have mild side effects, relevant in the prolonged treatment regimens and diminished bioavailability due to binding of metal ions. Moreover the resistance to fluoroquinolones is also on the rise, a characteristic of extensively drug resistant TB (XDR TB).

With these issues in mind, the present work focus on masking the acid moiety of fluoroquinolones, essential to the mode of action but also responsible for many of its side effects and metal chelating properties. A secondary objective was the modulation of the lipophilicity of the compounds. This was achieved by preparing esters as a prodrug of the fluoroquinolones levofloxacin and ciprofloxacin, with medium to long chain fatty alcohols.

The synthesis, stability in biological media and antibacterial activity were evaluated, the latter not only against *mycobacterium tuberculosis* but also against other clinically relevant bacterial species, since the parent compounds display a broad spectrum of activity. The biological results show a reduction in the antitubercular activity of the synthesized derivatives, probably due to deficient activation of the ester prodrug, nonetheless it was observed that the derivatives retain bioactivity against other fluoroquinolone-resistant bacteria, indicating a different mode of action.

Keywords: Esters; Fluoroquinolones; Prodrugs; Tuberculosis;

Introduction: Fluoroquinolones in the treatment of tuberculosis

Fluoroquinolones

Widely used antibacterial drugs

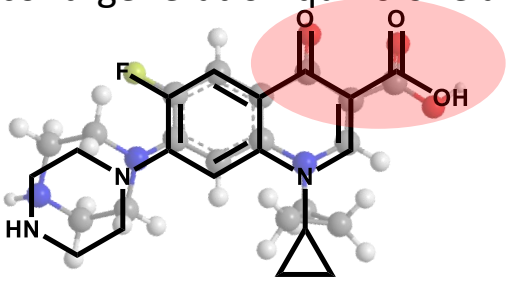
- ✓ Large spectrum of activity
- ✓ High potency
- X Side effects

Treatment of Tuberculosis (TB) and Multidrug-Resistant TB (MDR TB)

Targets

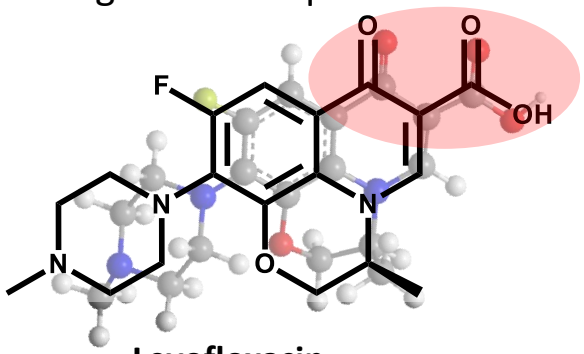
DNA gyrase

Second-generation quinolone antibiotic



Ciprofloxacin

Third-generation quinolone antibiotic



Levofloxacin

Mechanism of Action

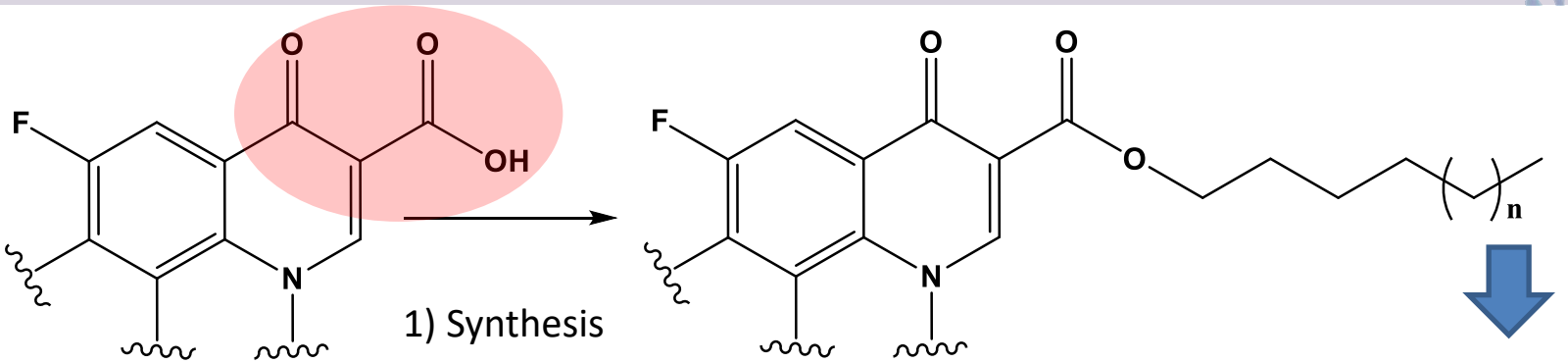
Inhibition of type II bacterial topoisomerase enzyme domains, **DNA gyrase** and **topoisomerase IV**

inhibition of DNA replication and transcription

Main mechanisms of resistance in TB

- X Mutations in gyrA and gyrB genes
- X Efflux systems

Introduction: Esters - a prodrug approach



Pro-drug transport

2) Chemical and Plasmatic stability

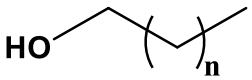
Pro-drug target activation

3) Mycobacterial hydrolysis

4) Antitubercular activity

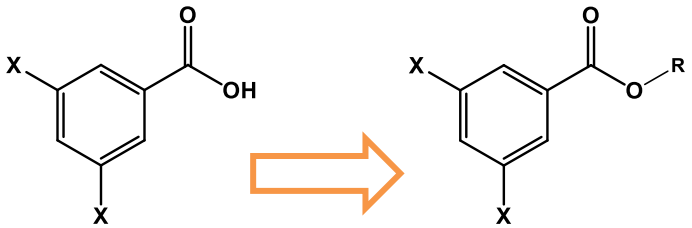
5) Broad antimicrobial activity

Fatty alcohols show antitubercular activity



n	MIC
0	1024
...	-
16	16

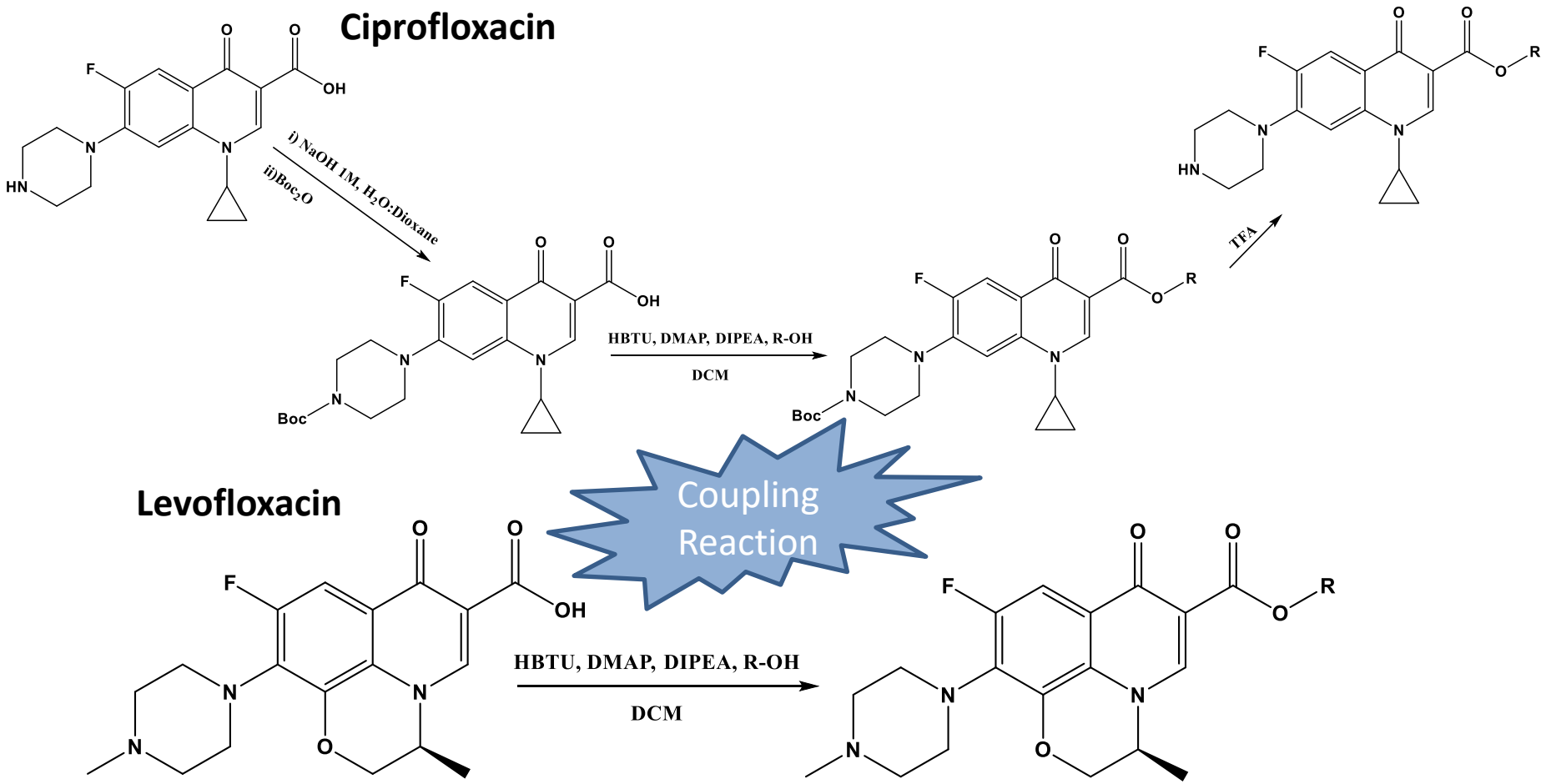
Ester approach increased activity of weak acids



160-80 mg/L

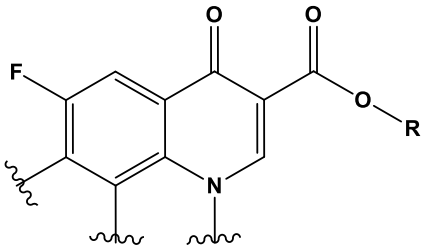
80 – 10 mg/L

Results and discussion: Synthesis



Results and discussion: Chemical and Plasmatic stability

Compounds	R	Degradation in PBS (%)	Degradation in human plasma (%)
1	C_6H_{13}	2,96	6,90
2	C_7H_{15}	1,42	10,82
3	C_8H_{17}	1,23	8,33
4	C_9H_{19}	0,55	5,06
5	$C_{10}H_{21}$	0,46	2,46
6	$C_{11}H_{23}$	0,19	2,25
7	$C_{12}H_{25}$	0	0,37
8	$C_{13}H_{27}$	0	0,35
9	$C_{14}H_{29}$	0	0,32
10	$C_{16}H_{33}$	0	0,24
11	C_6H_{13}	1,15	4,47



Chemically stable

Stable in plasma

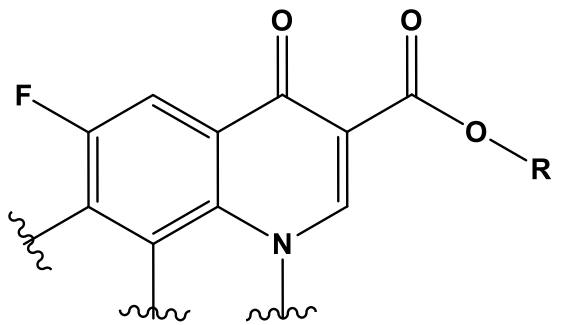
Results and discussion: Mycobacterial hydrolysis and antitubercular activity

Chemically stable ✓ → Stable in plasma ✓ → Mycobacterial activation ✗

Compounds	R	MIC (mg/L)	MBC (mg/L)
Levofloxacin	H	0,25	0,5
1	C_6H_{13}	2,5	5
2	C_7H_{15}	2,5	5
3	C_8H_{17}	2,5	5
4	C_9H_{19}	5	10
5	$C_{10}H_{21}$	10	20
6	$C_{11}H_{23}$	20	40
7	$C_{12}H_{25}$	10	40
8	$C_{13}H_{27}$	20	160
9	$C_{14}H_{29}$	20	40
10	$C_{16}H_{33}$	160	>160
Ciprofloxacin	H	<1.25	<1.25
11	C_6H_{13}	20	20
Isoniazid	-	0,06	0,06

No hydrolysis was observed against mycobacterium smegmatis homogenate

MIC/MBC values increased



Results and discussion: Broad spectrum antimicrobial activity

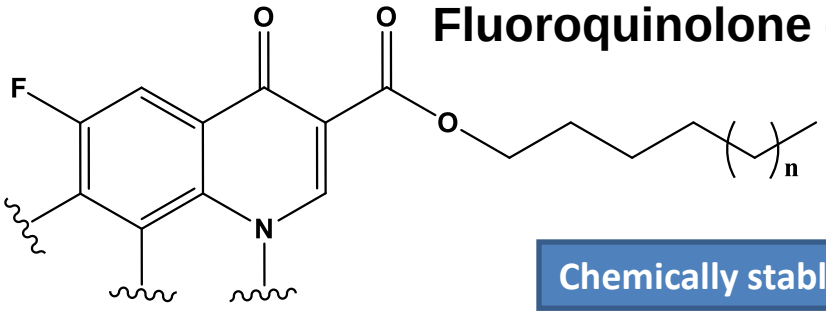
		Gram-positive bacteria				Gram-negative bacteria			
		Enterococcus faecalis ATCC 11420		Enterococcus faecalis ATCC 51299		E. coli ATCC 8739		Salmonella typhimurium ATCC 13311	
Compounds	Aliphatic chain	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
Levofloxacin	H	<4	8	<4	<4			<4	<4
1	C_6H_{13}	128	256	128	128	16	32	32	64
2	C_7H_{15}	128	256	64	128	32	32	32	32
3	C_8H_{17}	32	64	32	64	64	64	64	128
4	C_9H_{19}	16	8	16	16	64	64	32	128
5	$C_{10}H_{21}$	16	64	8	32	64	256	128	256
6	$C_{11}H_{23}$	32	256	16	128	128	256	128	256
7	$C_{12}H_{25}$	64	128	8	64	128	256	128	
8	$C_{13}H_{27}$	128	256	64	256	64	256	128	256
9	$C_{14}H_{29}$	128	256	64	256	256	256	128	
10	$C_{16}H_{33}$	256	256	128	256	256	256	265	
Ciprofloxacin	H	<4	8	<4	<4			<4	
11	C_6H_{13}	64	128	128	256	16	32	32	64
12	$C_{10}H_{21}$	32	64	32	64	32	256	32	128

Results and discussion: Broad spectrum antimicrobial activity

Compounds	Aliphatic chain	Staphylococcus aureus subsp. aureus Rosenbach ATCC ® 6538™ MIC (mg/L)	Staphylococcus aureus subsp. aureus Rosenbach ATCC ® 6538™ MBC (mg/L)	Vancomycin-resistant *Staphylococcus aureus* (VISA) CIP 106760 MIC (mg/L)	Vancomycin-resistant *Staphylococcus aureus* (VISA) CIP 106760 MBC (mg/L)	Staphylococcus aureus CIP 106414, ATCC 700699 MIC (mg/L)	Staphylococcus aureus subsp. aureus Rosenbach ATCC 43866 MIC (mg/L)	Staphylococcus aureus subsp. aureus Rosenbach ATCC 43866 MBC (mg/L)
Levofloxacin	H	<4	<4	128	256	<4	<4	
1	C_6H_{13}	64	128	256	256	64	64	256
2	C_7H_{15}	64	64	128	256	64	64	128
3	C_8H_{17}	32	32	32	64	32	16	32
4	C_9H_{19}	8	16	8	16		8	16
5	$C_{10}H_{21}$	8	16	8	256	8	8	16
6	$C_{11}H_{23}$	8	256	16	256		16	128
7	$C_{12}H_{25}$	16	256	128	256	64	8	256
8	$C_{13}H_{27}$	32	256	128	256		64	256
9	$C_{14}H_{29}$	32	256	128	256	128	32	256
10	$C_{16}H_{33}$	256	256	128	256	128	64	256
Ciprofloxacin	H	<4	<4	128	256	<4	<4	
11	C_6H_{13}	64	128	64	128	64	64	128
12	$C_{10}H_{21}$	16	32	16	32		32	64

Retains bioactivity against resistant bacteria!

Conclusions



Fluoroquinolone esters as a prodrug aproach

Chemically stable ✓ → Stable in plasma ✓ → Mycobacterial activation X

- Reduction in MIC values compared to parent compounds

Fluoroquinolone esters as a drug: new characteristics

- Diverse activity agains gram-positive and gram-negative bacteria
- Optimal chain lenght for gram-positive antibacterial action
- **Bioactivity observed against fluoroquinolone-resistant bacterial strain**

Diverse mode of action

Acknowledgments

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Thank you for your attention