THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par ***l'Université Toulouse III - Paul Sabatier***
Discipline ou spécialité **:** ***Chimie Biologie Santé***

Présentée et soutenue par ***Korolyov Alexander***
Le ***11 Avril 2011***

Dérivés lipophiles de la Ciprofloxacine et de la Lévofloxacine: Synthèse et évaluation de leurs activités antibactérienne, antimycobactérienne et antiprolifération.

JURY

MOREAU Pascale	***Professeur à l'Université Blaise Pascal, Clermont-Ferrand***
GREINER Jacques	***Chargé de Recherche au CNRS, Nice***
MARTIN VACA Blanca	***Professeur à l'Université Toulouse III***
GUIDETTI Brigitte	***Maître de Conférence à l'Université Toulouse III***

Ecole doctorale : ***Sciences de la Matière***
Unité de recherche : ***Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique (LSPCMIB) UMR 5068***
118, route de Narbonne 31062 TOULOUSE Cedex 9
Directeurs de Thèse **:** ***Brigitte Guidetti et Joëlle Azéma***
Rapporteurs : ***MOREAU Pascale et GREINER Jacques***

REMERCIEMENTS

- Laboratoire de Synthèse et Physicochimie de Molécules d'Intérêt Biologique, Michel Baltas
- Groupe de RMN Biomédicale, Myriam Malet Martino
- Les directrices Brigitte Guidetti et Joëlle Azéma
- Les équipes de service de RMN, spectroscopie de mass, HPLC
- Les collaborateurs: Christine Roques, Laboratoire de Microbiologie Industrielle, Toulouse; Patricia Constant et Mamadou Daffé, Laboratoire de Mécanismes Moléculaires des Infections Mycobactériennes, Toulouse; Robert Kiss, Laboratoire de Toxicologie, Bruxelles

LIST OF ABBREVIATIONS

2-TT, 2-thiazoline-2-thiol.
B. fragilis, Bacteroides fragilis.
Boc, t-butyloxycarbonyl.
CAP, community-acquired pneumonia.
CC_{50}, concentration that reduces cell viability by 50%.
CIP, ciprofloxacin.
CLI, clinafloxacin.
DCC, N,N'-dicyclohexylcarbodiimide.
DIPEA, diisopropylethylamine.
DMSO, dimethylsulfoxide.
E. coli, Escherichia coli.
EARSS, european antimicrobial resistance surveillance system.
EC_{50}, half maximal effective concentration.
FLE, fleroxacin.
FQ, fluoroquinolone.
GAT, gatifloxacin.
GEM, gemifloxacin.
GRE, grepafloxacin.
HOBt, 1-hydroxybenzotriazole.
HPLC, high-performance liquid chromatography.
IC_{50}, concentration of compound required for 50% inhibition effect.
ITQ, isothiazoloquinolones.
K. pneumoniae, Klebsiella pneumoniae.
LEV, levofloxacin.
M. smegmatis, Mycobacterium smegmatis.
M. tuberculosis, Mycobacterium tuberculosis.
MBC, minimum bactericidal concentration.
MDR, multi-drug resistant.
MDRSA, multidrug resistant Staphylococcus aureus.
MIC, minimum inhibitory concentration.
MOX, moxifloxacin.
MPC, mutant prevention concentration.
MRSA, methicillin-resistant Staphylococcus aureus.
MTD, maximum tolerated dose.
MTT, 3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide.
NAD, nadifloxacin.
NAL, nalidixic acid.
NHS, *N*-hydroxysuccinimide.
NMR, nuclear magnetic resonance.
NOR, norfloxacin.
OFL, ofloxacin.
P. aeruginosa, Pseudomonas aeruginosa.
PAZ, pazufloxacin.
PBS, phosphate buffer saline.
PEF, pefloxacin.
QRDR, quinolone-resistance determining region.
S. aureus, Staphylococcus aureus.
S. pneumoniae, Streptococcus pneumoniae.
S. pyogenes, Streptococcus pyogenes.
SAR, structure-activity relationship.
SPA, sparfloxacin.
TB, tuberculosis.
TBAI, tetrabutylammonium iodide.
TCA, trichloroacetonitrile.
TMP, 2,4,6-trimethylpyridine.
TPP, triphenylphosphine.
TRO, trovafloxacin.
ULI, ulifloxacin.
UTI, urinary tract infections.
UV, ultraviolet.
VRSA, vancomycin-resistant Staphylococcus aureus.
WHO, world health organization.
XDR, extensively drug-resistant.

Contents

INTRODUCTION GÉNÉRALE

Le ciprofloxacine (CIP) et la lévofloxacine (LEV) sont des antibiotiques de la famille des fluoroquinolones (FQs). Ce sont des agents à large spectre qui ciblent les topoisomérases bactériennes de type II. Certaines FQs ont également été approuvées comme médicaments de seconde ligne pour le traitement des tuberculoses multirésistantes. Même si la recherche de nouvelles FQs antibactériennes a déjà été largement développée, ces molécules sont encore un sujet d'étude en particulier à cause de la demande continue de nouveaux composés actifs contre les agents pathogènes résistants. Des récentes études structure-activité ont montré les avantages de la modification en position C-7 des FQs conduisant à une activité accrue contre des bactéries à Gram-positif, en particulier *Staphylococcus aureus (S. aureus)* résistant à la méticilline.

Etant donné les similitudes structurelles existant entre les topoisomérases de type II chez les procaryotes et les eucaryotes, les quinolones ont, plus récemment, été étudiées pour leur capacité à inhiber la topoisomérase II mammifère. Des études ont montré que des modifications des quinolones en positions C-7 et C-3 peuvent conduire à une nouvelle classe d'agents anti-tumoraux. Compte tenu de ce qui précède, nous nous sommes intéressés à développer de nouveaux dérivés des FQs. Deux voies ont été envisagées : la modification en position C-7 de la CIP et la modification sur l'acide carboxylique en C-6 de la LEV.

Le premier chapitre de cette thèse sera consacré à une revue bibliographique relative à l'état actuel des recherches dans le domaine des FQs en tant qu'agents antibiotiques, antimycobactériens et antitumoraux. Un accent particulier sera mis sur les composés modifiés en C-7 et en C-3 (ou C-6) du pharmacophore. Cela permettra une meilleure compréhension du mécanisme d'action et de résistance ainsi que la possibilité de justifier nos choix de modifications structurales, et d'expliquer les résultats des tests biologiques.

Le deuxième chapitre décrit la synthèse des nouveaux dérivés de la CIP et la LEV. Nous passerons en revue les voies de synthèse déjà essayées et des défis rencontrés. Des études d'optimisation et la comparaison des différentes méthodes mises au point seront mentionnés ainsi que les conditions optimales dans chacune des séries de composés.

Dans le troisième chapitre nous discuterons les diverses activités biologiques obtenues pour les dérivés synthétisés. L'influence du type de substitution, de la structure dimère (ou monomère), du noyau quinolone sur l'activité antibactérienne, antimycobactérienne et antiproliférative des composés sera présentée. Finalement, nous essayerons de généraliser les facteurs régissant l'activité et la spécificité contre les différentes cibles biologiques.

CHAPITRE I BIBLIOGRAPHIE

1. Les quinolones antibactériennes

1.1 Généralités

Pour des raisons de compréhension, avant de détailler les propriétés antibactériennes des quinolones, certains concepts fondamentaux de microbiologie seront présentés. La plupart des bactéries peuvent être classées dans deux groupes selon la coloration de Gram. Cette technique permet de mettre en évidence les propriétés de la paroi bactérienne, et d'utiliser ces propriétés pour les distinguer et les classifier (à Gram-positif et à gram-négatif). Des exemples représentatifs des bactéries à Gram-négatif sont: *Escherichia coli (E. coli)* et *Pseudomonas aeruginosa (P. aeruginosa)*. Des bactéries à Gram-positif d'importance médicale incluent: *S. aureus* et *Streptococcus pneumoniae (S. pneumoniae)*. Une autre classification est basée sur la nécessité (ou pas) de la présence de dioxygène dans le milieu de culture des microorganismes: on parle alors de bactéries aérobies ou anaérobies.

1.2 Histoire

Le motif quinolone est apparu il y a 50 ans[1] et c'est toujours l'une des structures les plus courantes dans le domaine des molécules biologiquement actives. Les quinolones sont des agents anti-infectieux totalement synthétiques dont la structure moléculaire se compose de plusieurs systèmes cycliques (hétérocycliques). Les plus importants, ainsi que leurs systèmes de numérotation sont illustrés sur la **Figure 1**.

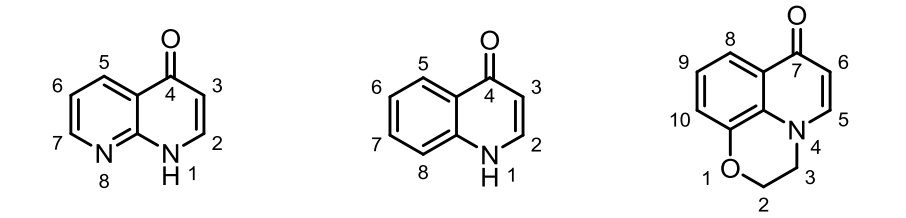


Figure 1 Structures et systèmes de numérotation des quinolones les plus importantes

Les quinolones de première génération ont été reconnues comme des antibiotiques dont le spectre d'activité était limité aux bactéries à Gram négatif aérobies. Au fil des années, de nouvelles quinolones ont vu le jour avec l'introduction en 1980 de l'atome de fluor en position C-6. Les fluoroquinolones (FQs) ne sont pas seulement plus actives que les agents précédents contre les bactéries à Gram-négatif, mais leur spectre a été étendu successivement aux bactéries à

Gram positif et aux anaérobies cliniquement importantes. Les FQs sont devenues l'un des groupes les plus utilisés des antibiotiques à large spectre[2].

La famille des quinolones antibactériennes est généralement divisée en générations, dans le paragraphe suivant nous allons utiliser la classification basée sur le spectre antimicrobien[3].

1.2.1 Première génération

Les quinolones sont issues des premiers travaux de George Lesher et ses collaborateurs en 1962[1]. L'activité antimicrobienne de l'acide nalidixique (NAL, **Tableau 1**) a été étudiée sur un panel de micro-organismes, elle a révélé son activité contre les agents pathogènes à Gram négatif comme *E. coli* et *Klebsiella pneumoniae (K. pneumoniae)*. Cependant, plusieurs inconvénients tels qu'une pharmacocinétique défavorable, des effets indésirables, et une faible activité contre les bactéries à Gram-positif et anaérobies, ont limité les indications de NAL aux infections des voies urinaires non compliquées (IVU)[4]. Néanmoins, ce composé a stimulé un intérêt croissant pour cette classe et a donné lieu à des développements ultérieurs.

1.2.2 Deuxième génération

Les quinolones de deuxième génération sont marqués par l'introduction d'un atome de fluor en position C-6, ce qui a grandement amélioré leur spectre d'action et augmenté l 'absorption des médicaments par les cellules bactériennes[5]. Ce groupe est généralement représenté par deux sous-classes.

La classe I est représentée par la norfloxacine (NOR, **Tableau 1**) et la péfloxacine (PEF, **Tableau 1**) qui possèdent une activité remarquablement augmentée contre les bactéries à Gram-négatif et une activité importante contre certains agents pathogènes à Gram-positifs, comme *S. aureus* sensible à la méticilline. Pourtant, cette classe présente encore des inconvénients majeurs par exemple, une distribution tissulaire faible[6].

La classe II est marqué par l'émergence de la ciprofloxacine[7] (CIP, **Tableau 1**), dérivant de NOR par le remplacement du substituant N-1 éthyl par cyclopropyl. CIP possède l'une des activités les plus puissantes contre une large gamme d'organismes à Gram-négatif, une augmentation d'activité contre les bactéries à Gram-positif et les bactéries atypiques[8]. Un autre représentant de cette classe est l'ofloxacine (OFL, **Tableau 1**) qui introduit un pont entre les positions N-1 et C-8 du noyau quinolone, conduisant au squelette 1,4-benzoxazine. En raison de la pharmacocinétique améliorée[9] (biodisponibilité et distribution tissulaire) et du large spectre d'activité, la LEV et la CIP sont devenues des antibiotiques par voie orale largement utilisés et ont élargi les indications des FQs pour inclure : les infections gastro-intestinales, génitales,

osseuses et ostéo-articulaires. Cependant, les molécules de cette classe II n'ont pas d'activité anti-streptocoques à Gram-positif telles que *S. pneumoniae*. Par ailleurs, l'utilisation importante (et parfois abusive) du CIP a provoqué l'émergence de souches résistantes dans les hôpitaux et les communautés[10].

1.2.3 Troisième génération

La séparation des énantiomères de OFL a montré que l'isomère *R* est presque inactif alors que l'isomère S est responsable de l'activité du racémate[11]. L'isomère *S* a été nommé lévofloxacine (LEV, **Tableau 1**) et a présenté un niveau élevé d'activité contre les staphylocoques et les anaérobies, y compris les souches résistantes à la méticilline. Toutefois, la LEV a présenté une activité légèrement inférieure à CIP contre les agents pathogènes à Gram-négatif, y compris *P. aeruginosa*[12]. Les autres représentants de cette classe, la gatifloxacine (GAT, **Tableau 1**) et la moxifloxacine (MOX, **Tableau 1**) ont également une bonne activité contre les souches à Gram-positif [13], y compris les souches résistantes à la CIP[14]. Les 8-OMe FQs ont été introduites avec l'intention de diminuer la fréquence de sélection des mutants[15]. Les concentrations sériques élevées et les demi-vie plus longues ont permis le dosage une seule fois par jour pour les représentants de la troisième génération[16]. Ces caractéristiques ont élargi la gamme de leurs indications à la pneumonie acquise en communauté (PAC) en comparaison avec les générations précédentes.

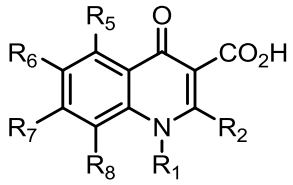
1.2.4 Quatrième génération

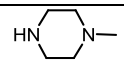
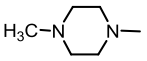
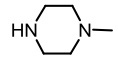
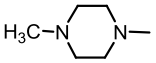
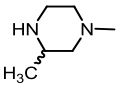
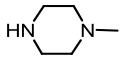
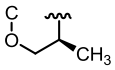
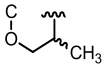
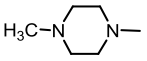
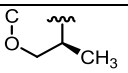
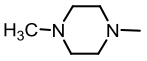
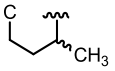
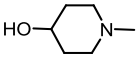
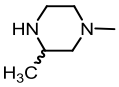
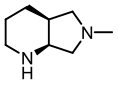
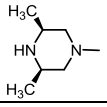
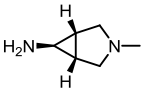
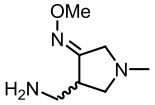
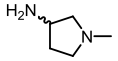
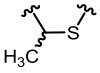
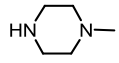
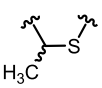
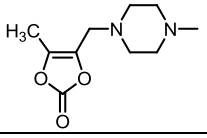
Les quinolones de quatrième génération sont les composés les plus puissants avec un spectre large et un haut degré d'activité. Avec l'augmentation de l'activité anti-Gram-positif ces dérivés possèdent une activité considérablement augmentée contre les anaérobies[17] et les bactéries à Gram-négatif, y compris *P. aeruginosa*[18]. L'élargissement du spectre et l'augmentation du degré d'activité antibactérienne a permis de nouvelles indications aux FQs de 4ème génération dans le traitement des infections intra-abdominales. Du point de vue de la structure chimique, en plus des changements de substituants en position C-7, ces agents comme la trovafloxacine (TRO) et la gémifloxacine (GEM,) sont des dérivés de type 1,8-naphtyridine, similaires à NAL, tandis que ulifloxacin[19] (ULI, **Tableau 1**) qui est utilisé sous la forme de prodrogue (prulifloxacin[20], PRU, **Tableau 1**) comporte une annélation originale entre le N-1 et le C-2 de la quinolone.

Pour conclure, au cours des différentes modifications structurales les quinolones ont considérablement augmenté leur spectre antibactérien et leur degré d'activité. Ces

caractéristiques, combinées à l'amélioration de leur pharmacocinétique ont permis un élargissement de leur indication pour le traitement des infections urinaires, des voies respiratoires et intra-abdominales. Toutefois, les problèmes de toxicités ont entraîné le retrait de plusieurs quinolones commercialisées. Par ailleurs, l'apparition de phénomènes de résistance aux anciennes quinolones a également perturbé le développement de cette classe d'antibactériens. Dans le prochain paragraphe, nous donnerons un aperçu du mécanisme d'action et de résistance des FQs, ce qui nous aidera à rationaliser nos synthèses et à expliquer nos résultats biologiques.

Tableau 1 Structures représentatives des différentes générations de quinolone



Génération	*Nom*	*R_8*	*R_1*	*R_2*	*R_5*	*R_6*	*R_7*
1	NAL	N	Et	H	H	H	CH_3
2 Class I	NOR	C-H	Et	H	H	F	
	PEF	C-H	Et	H	H	F	
	ENO	N	Et	H	H	F	
	FLE	C-F	CH_2CH_2F	H	H	F	
	LOM	C-F	Et	H	H	F	
2 Class II	CIP	C-H		H	H	F	
	PAZ			H	H	F	
	OFL			H	H	F	
3	LEV			H	H	F	
	NAD			H	H	F	
	GRE	C-H		H	CH_3	F	
	MOX	C-OMe		H	H	F	
	GAT	C-OMe		H	H	F	
	SPA	C-F		H	NH_2	F	
4	TRO	N		H	H	F	
	GEM	N		H	H	F	
	CLI	C-Cl		H	H	F	
	ULI	C-H			H	F	
	PRU	C-H			H	F	

1.3 Mechanism of action and resistance

1.3.1 Targets for antibacterial quinolones

DNA gyrase was first found in Gram-negative organisms. For many years it was thought to be the only quinolone target[21]. However, in 1990 DNA topoisomerase IV, a homolog of gyrase, was discovered[22] and it was demonstrated that this enzyme is a primary target of FQ in Gram-positive *S. aureus*[23]. Both gyrase and topoisomerase IV are composed of two subunits (GyrA and GyrB in gyrase, ParC and ParE* in topo IV) assembled into a functional heterotetramer i.e. A_2B_2 and C_2E_2 respectively. Both enzymes are type II topoisomerases as they cleave both strands of DNA double-helix. After the passage of unmodified strand (T-segment) through the temporary gap (G-segment) they promote religation of broken strands[24] (**Figure 2**).

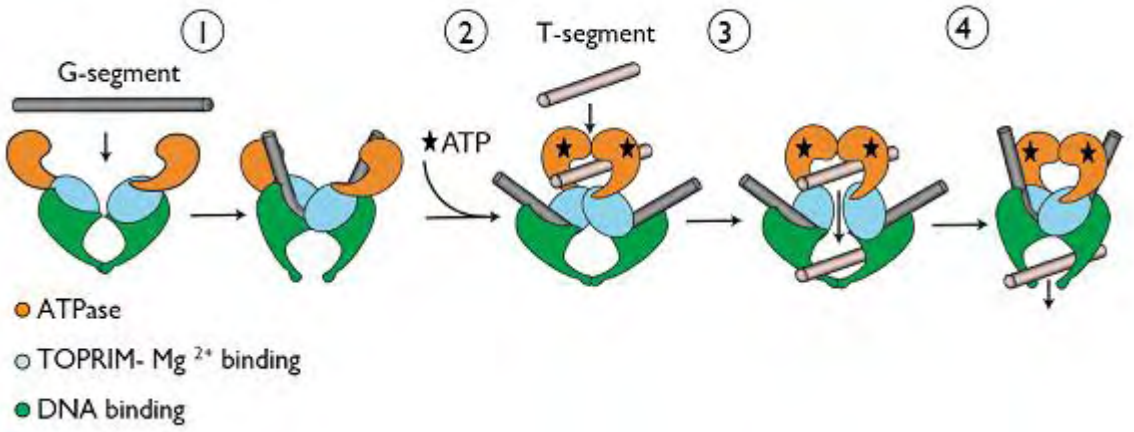


Figure 2 Two-gate mechanism of type II topoisomerase action (From ref [24] with permission of Nature Publishing Group)

In this way topoisomerases manage DNA topology: they could resolve knots and release superhelical tensions which are produced during DNA transcription and replication. In the cell DNA gyrase facilitates DNA unwinding in front of the moving replication fork and topoisomerase IV has a specialized function in mediating the decatenation of interlocked daughter chromosomes behind the replication fork (**Figure 2**). In this way these enzymes are vital for the normal cell's functioning.

*Historically in *S. aureus* these two subunits of topoisomerase IV: were named GrlA and GrlB

Quinolones bind the DNA-enzyme complex where DNA is kept in the intermediate stage of double helix passing in which both DNA strands are cut. Ternary complex forms a barrier to the moving replication fork and arrests it 10 base-pairs upstream the DNA cleavage sites[25]. However due to reversibility of the quinolone-enzyme-DNA complex formation, DNA inhibition cannot be the cause of immediate cell death and fails to correlate with rapid cell death in terms of kinetics, quinolone concentration. The most straightforward model is that drug-enzyme-DNA complexes block bacterial growth (determined by Minimum inhibitory concentration, MIC - is the lowest concentration of drug that inhibits more than 99% of the bacterial population) while the release of double-stranded DNA breaks was proposed to be the cause of lethal consequences[26].

1.3.2 Structure of ternary complex

For a long time the structure of the ternary complex remained unresolved and only tentative models based on structure-activity relationship (SAR) and mutations studies were proposed for the quinolone-DNA-topoisomerase interaction. Recently *Lapogonov et al.* finally resolved the crystal structure of the quinolone-DNA cleavage complex of the topoisomerase IV from *S. pneumoniae*[27]. Two molecules of MOX are intercalated in the gap between -1 and +1 nucleotides at the two ends of DNA double-strand cut (**Figure 3**).

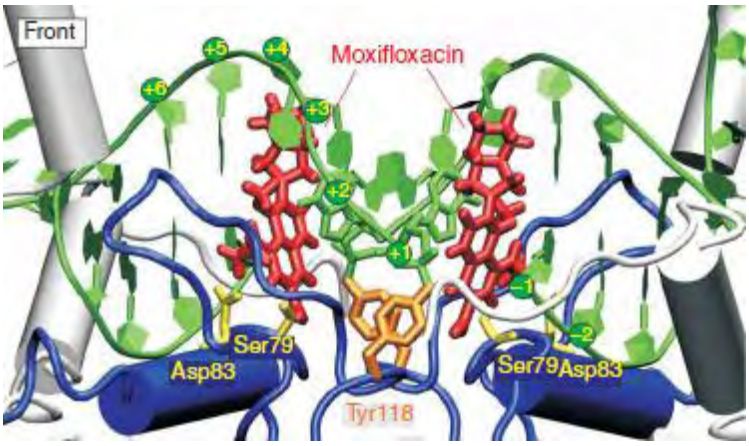


Figure 3 Two molecules of MOX bound to the *S. pneumoniae* topoisomerase IV-DNA cleavage complex. (From ref [27] with permission of Nature Publishing Group)

The bulky substituent at C-7 position is positioned next to the DNA bases at +4 and +5 positions on the opposite DNA strand and projects into large solvent-accessible volume above the DNA cut (**Figure 3**). This result is consistent with SAR studies showing that large variations

at C-7 position are accepted (see section 2.3.2). The N-1 cyclopropane ring is located close to ParC α-helix residue Ser79 and Asp83, whose mutation is responsible for quinolone resistance [28](**Figure 4**).

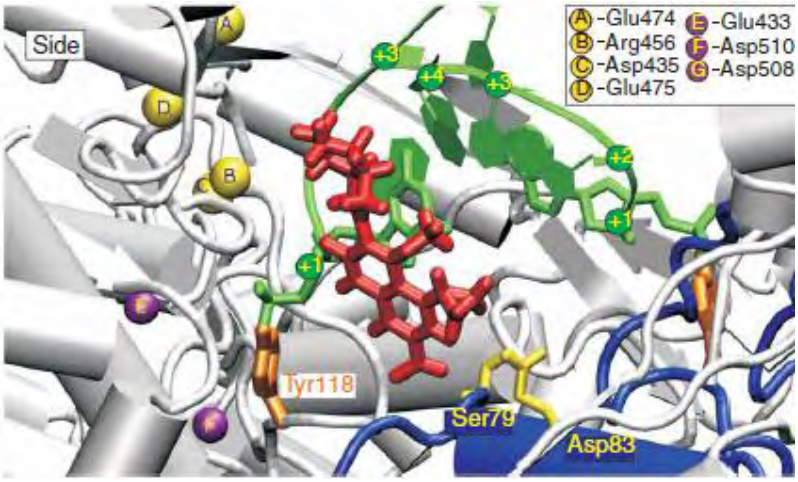


Figure 4 MOX bound to *S. pneumoniae* topoisomerase IV-DNA cleavage complex. Quinolone-enzyme interactions (From ref [27] with permission of Nature Publishing Group)

The fluorine atom at C-6 position is situated far from the protein chain and DNA strand so its influence on the quinolone potency might be explained by altering the electron density of the heterocyclic core. Studies have shown that by fine tuning of other substituents one could achieve high level of potency without fluorine atom at C-6[29]. The 3-carboxy and 4-keto groups of the quinolone are located closest to the ParC α-helix Gly77, Asp78 residues which are possible partners for hydrogen bonding; but far away from the magnesium binding side of the ParC TOPRIM (topoisomerase-primase) domain, though direct metal chelation is questioned.

1.3.3 Resistance to quinolones

Over the 30 years that have elapsed since the introduction of FQs, resistance to these agents has become common and widespread. A number of reports of the European Antimicrobial Resistance Surveillance System (EARSS) showed gradual increase of the part of FQ-resistant pathogens in Europe: for example from 9% (2001) to 20% (2009) for *E. coli*[30]. These facts show the growing threat of emerging non-susceptibility to older quinolones and the constant need for the development of new agents.

1.3.3.1 Modifications at the target site and target preference

The most important mechanism of FQ resistance is mediated by amino acid substitutions in the target enzymes. This type of resistance arises stepwise. In several species, the first-step mutations occur in the *gyrA* and occasionally in *gyrB* (genes encoding GyrA and GyrB subunits of DNA gyrase) while in others they occur in *parC* and less often in *parE* (genes encoding ParC and ParE subunits of topoisomerase IV). Genetic evidences of this type are often used to prove the target preference of the FQ.

In Gram-negative species, GyrA is the primary target of 4-quinolones, as the first mutations conferring resistance occur in GyrA and single ParC mutations do not change the MIC of quinolones for *E. coli*[31]. The whole set of the most frequently occurred mutation was named "quinolone-resistance determining region" (QRDR) which is located between amino acids positions 67 and 106 in GyrA subunit of *E. coli* gyrase[32]. These residues are situated in the proximity to the putative quinolone binding pocket in the enzyme-DNA cleavage complex and thus reduce the drug affinity to the complex of modified enzyme and DNA. Amino acid positions 447 and 426 in GyrB subunit were found to be responsible for the low-level resistance[33].

In Gram-positive species, the situation with target preference is not so evident; both topoisomerase IV and gyrase may be the targets with different degree of preference.

Research with a large number of *S. aureus* isolates showed that the Ser80Phe alteration in GrlA and Ser84Leu change in GyrA are the principal ones and both mutations in DNA gyrase and topoisomerase IV are needed for high-level resistance[34].

The target preference in *S. aureus* varies between quinolones and according to studies of *Takei et al.*[35] these agents can be divided into three categories. The first type includes NOR, ENO, fleroxacin (FLE), CIP, OFL and LEV (**Tableau 1**); which have greater MICs for *grlA* mutant strains (*grlA* is the gene encoding GrlA subunit of topo IV) than for *gyrA* mutant strains (*gyrA* is the gene encoding GyrA subunit of gyrase). These quinolones displayed lower half maximal inhibitory concentration (IC_{50}) values (the concentration of drug required for 50% inhibition of enzyme activity *in vitro*) for purified topoisomerase IV from wild-type strain than IC_{50} values for DNA gyrase. These results suggest the preferential topoisomerase IV inhibition by this group of quinolones.

The second type includes sparfloxacin (SPA) and nadifloxacin (NAD) **(Tableau 1)**. These quinolones showed reversed order of activity: greater MICs for *gyrA* than for *grlA* mutants and lower IC_{50} values for DNA gyrase. These results suggest the preferential DNA gyrase inhibition by these analogs.

The third type comprises GAT, pazufloxacin (PAZ), MOX and clinafloxacin (CLI) (**Tableau 1**). In this group, similar values of MICs and IC_{50} for both targets were observed. It has been suggested that antibacterial activity of these compounds are mediated by dual-inhibition of both DNA gyrase and topoisomerase IV.

The most frequent resistance mutations in *S. pneumoniae* comprise alterations in ParC (Ser79Tyr/Phe, Ala84Thr) and in GyrA (Ser81Tyr)[36], which are analogous to the previously mentioned in other species. Also single mutations in ParC conferred only low-level resistance while for the high-level resistance both DNA gyrase and topoisomerase IV have to be altered[37]. The target preference at *S. pneumoniae* is also dependent on type of quinolone. Comparing IC_{50} of purified enzymes and single-step mutations it was proposed that for CIP, LEV, NOR, TRO[38] topo IV is a primary target, while for SPA, GAT[38], grepafloxacin[39] (GRE, **Tableau 1**) it is the DNA gyrase; CLI seems to be equipotent against both enzymes[40].

1.3.3.2 Efflux mediated resistance

The second mechanism of resistance in bacteria is an active drug efflux by overexpression of certain efflux pumps. These are transmembrane proteins which are responsible for the active transport of different molecules. Efflux pump mechanisms probably have a preexisting physiological role, protecting the bacillus against high intracellular levels of toxic molecules. In addition, efflux pumps maintain cellular homeostasis and physiological balance through transport of the toxins or metabolites to the extracellular environment[41]. Specific gene *norA* was found to confer quinolones resistance in *S. aureus*[42]. The study of relative contribution of efflux mediated resistance by selective inhibition of multidrug efflux pumps showed that hydrophilic quinolones are more susceptible to the active transport while more hydrophobic MOX, SPA and TRO are less affected by this type of resistance[43]. Bulky substituents and large molecular weight of compounds should also reduce their transport by efflux pumps.

1.3.3.3 Plasmid mediated resistance

The third and recently discovered mechanism of resistance is associated with intracellular plasmids. A multiresistance plasmid was recently discovered that encodes transferable resistance to quinolone[44]. The plasmid-quinolone resistance gene was termed *qnr* and the corresponding protein Qnr was shown to protect *E. coli* DNA gyrase from the inhibition by CIP. It was hypothesised that protective action of Qnr results from the formation of Qnr–gyrase complex which occurs before the formation of the cleavage complex with DNA. It is noteworthy that

plasmids are extrachromosomal genetic elements, thereby granting plasmid mediated resistance a high degree of mobility which poses risks of the resistance spreading among different organisms.

In this paragraph, we have shown that bacterial type II topoisomerases are intracellular targets of FQs that turn these enzymes into cellular poisons. Between different resistance mechanisms, mutations at the target site are the most important. However, the relative activity of FQs is influenced by their intracellular concentration in the cell. The latter is determined by the ability of drug molecules to penetrate the cellular wall and will be discussed in detail in the next paragraph.

1.3.4 Penetration into bacterial cell

Penetration of quinolones into bacterial cell is tightly related with cellular efflux/influx balance. In the case of extracellular flow, there are evidences for the presence of active multidrug efflux in resistant strains, as we have described in the section 1.3.3.2. By contrast, quinolone influx seems to be energy-independent, governed by the passive diffusion[45].

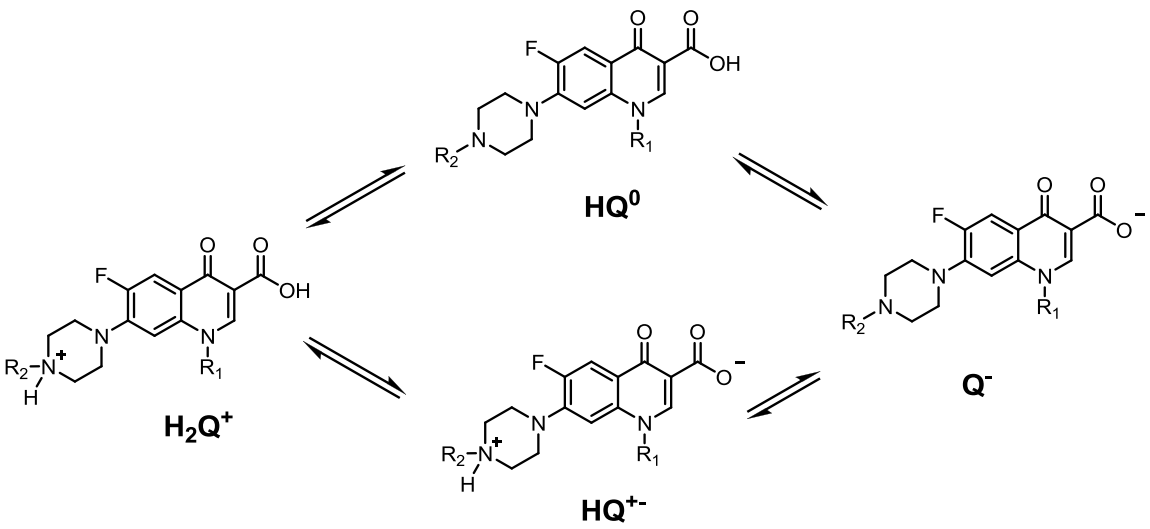


Figure 5 Protonation equilibria of FQ

To date, there are numerous studies that reveal correlations between lipophilicity, molecular weight, acidity/basicity and FQ uptake in different organisms. Because quinolones present both acidic and basic functionality, these molecules could exist in four different microspecies in aqueous solution: cationic H_2Q^+, neutral HQ^0, zwitterionic HQ^{+-} and anionic Q^- (**Figure 5**). Several experimental evidences testify that in Gram-positive organisms with a single cell membrane FQ transferred in the neutral HQ^0 form by passive diffusion through phospholipid bilayer membrane; more lipophilic analogs should have higher transport rates[46]. In Gram-

negative species penetration is complicated by the presence of the additional lipopolysacharide outer membrane, which is 2 orders of magnitude less permeable than ordinary phospholipid bilayer towards lipophilic compounds (**Figure 6**).

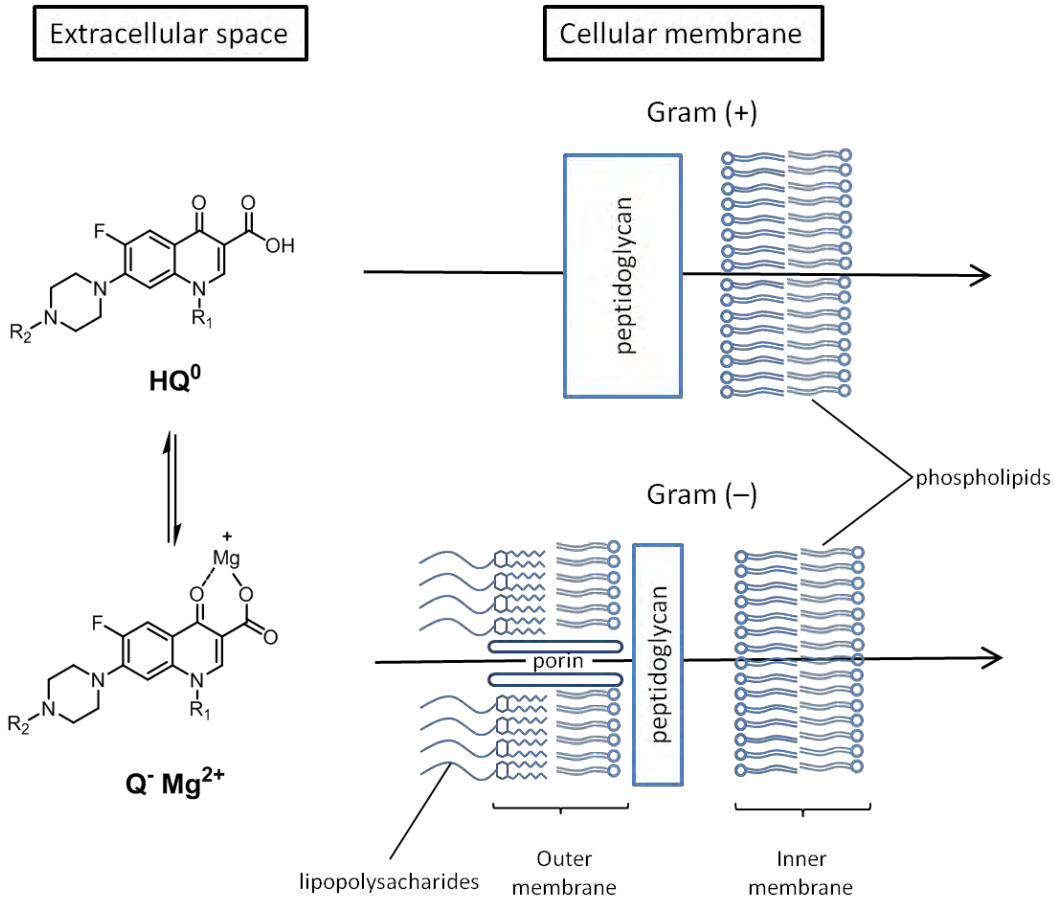


Figure 6 Simplified cell wall structures in Gram-positive and Gram-negative bacteria and their penetration by FQ.

In this case the main part of the FQ influx assisted through the porin channels. Porins are beta-barrel proteins that cross an outer membrane and act as a pore with size-limited permeability (with slight cation selectivity). So only relatively small and hydrophilic molecules can freely penetrate them[47]. Chelation of FQ with magnesium ions would provide hydrophilic positively-charged species which are thought to be responsible for the FQ influx in Gram-negative species[45].

In this paragraph, we have described the modern understanding of quinolone action and resistance which will help us to understand some of structure-activity dependencies in the next paragraph.

1.4 Structure-activity relationships

The history, development and SARs of the quinolones have been extensively reviewed[48]. Quinolone SARs are usually examined position by position so in this section we will introduce general findings; then we will focus on the C-3 and C-7 positions which are relevant to our work.

1.4.1 General trends

As synthetic antibacterial agents, FQ consist of the main 3-carboxy-4-pyridone pharmacophore and, as auxopharmacophore, a fused aromatic ring with attached substituents which serves to modulate the potency and antibacterial spectrum (**Figure 7**).

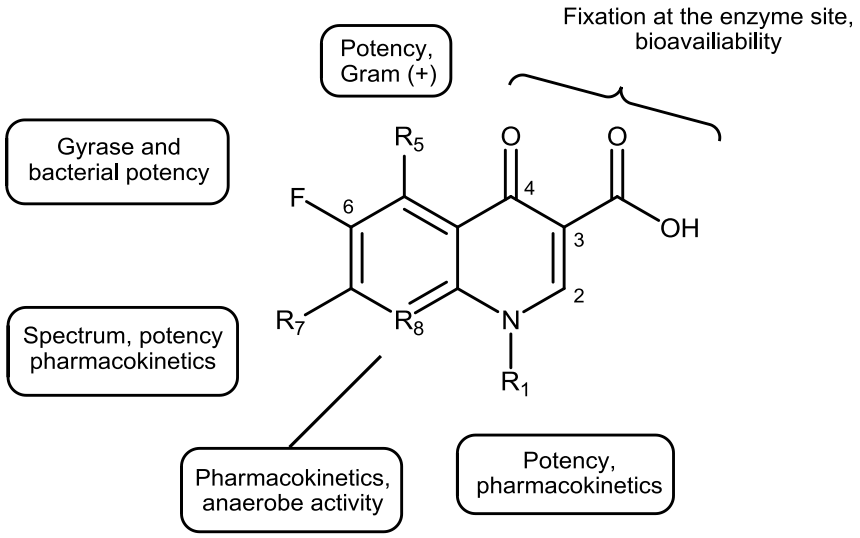
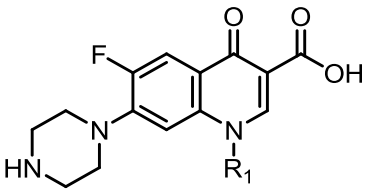


Figure 7 General trends in FQ SAR

SAR studies in the early era suggested that optimal groups to be attached to the N-1 position should be relatively small and hydrophobic[5]. Apart from the N-1 ethyl substituent in NOR or cyclopropyl group in CIP, some aryl groups at N-1 could improve Gram-negative spectrum of activity in comparison to NOR[49] (**Table 2**).

Table 2 Antibacterial activity of some N-1 substituted quinolones: alkyl and aryl substituents

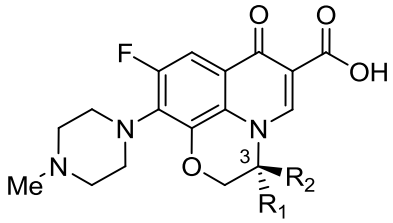


R_1	*MIC, μM*	
	Gram (–)	*Gram (+)*
	E. coli[a]	*S. aureus*[b]
CH_3	1.26	20.2
CH_2CH_3 (NOR)	0.16	1.22
$CH_2CH_2CH_3$	0.60	4.68
CH_2CH_2F	0.30	4.64
CH_2CH_2OH	1.16	4.65
	E. coli[c]	*S. aureus*[d]
CH_2CH_3 (NOR)	0.31	2.44
C_6H_5	0.56	1.06
4'-FC_6H_4	0.13	0.54

Data from ref [5, 49] [a]*E. coli* NIHJ JC-2 strain [b]*S. aureus* 209P strain [c]*E. coli* Juhl strain [d]*S. aureus* ATCC 6538P strain

Further research for possible N-1 substituents has led to preparation of tricyclic quinolone ring systems in which a new saturated ring is formed to give 1,8-bridged quinolones – LEV and OFL. Studies have shown that there is a stereochemical preference for the methyl at the bridging 3-position[50]. Stereochemistry of LEV gives approximately 12 fold increase in DNA gyrase inhibitory activity in comparison to its enantiomer, while ratio for the *in vitro* MICs varies in 31-125 fold increase and is much greater than expected from gyrase inhibition[50b] (**Table 3**)

Table 3 Antibacterial activity of 1,8-bridged quinolones: Stereoselectivity

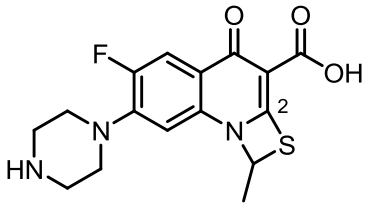


Name	*R_1*	*R_2*	*MIC(μM)*			*IC_{50} (μM), DNA gyrase* [a]
			Gram (+)		*Gram (–)*	
			E. coli[b]	*P. aeruginosa*[c]	*S. aureus*[d]	
OFL (racemate)	H/CH_3	H/CH_3	0.14	0.55	1.1	2.10
LEV	H	CH_3	0.07	0.28	0.55	1.05
-	CH_3	H	2.16	17.3	69	13.0

Data from ref [50] [a]Corresponds to 50% inhibition of DNA gyrase supercoiling activity isolated from *E. coli* KL 16 [b] *E. coli* KL 16 strain [c]*P. aeruginosa* 32122 strain [d]*S. aureus* 209P strain

The C-2 position is also close to the fixation site, so for a long period of time hydrogen atom was the only successful substituent. However, an example of C-2 modified potent compound is ULI (**Table 4**) with a broad-spectrum activity comparable to that of CIP[19].

Table 4 Ulifloxacin antimicrobial activity



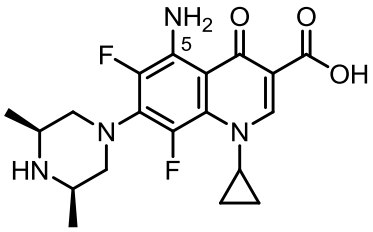
Compound	*MIC_{50}, μM*[a]		
	Gram (–)		*Gram (+)*
	E. coli(43[b]*)*	*P. aeruginosa(42*[b]*)*	*S. aureus(37*[b]*)*
ULI	0.29	1.13	2.26
CIP	0.15	0.60	1.18

Data from ref [19] [a]Compounds were tested against collection of clinical isolates; MIC_{50} is MIC for 50% of isolates tested. [b]Total number of isolates in a collection

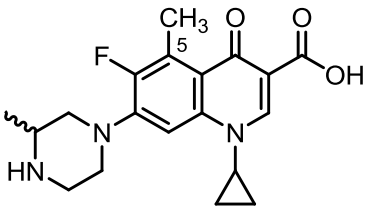
Initial findings showed that modifications in the 4-pyridone ring render significantly less active compounds. There are no analogs with alterations at C-3 and C-4 positions that reached noticeable degree of activity. From the structural data of the quinolone-DNA-enzyme complex, it was proposed that these positions take part in the quinolone fixation at the active site of enzyme.

The C-5 position is left unsubstituted in the large part of commercial quinolones. It is thought to influence potency and provide additional Gram-positive activity. This position demonstrates important phenomena of the different substituent interaction within quinolone heterocyclic core. While C-5 substitution itself induces chromosomal injury the use of finely tuned combination of 5-NH_2 and C-8 substituents results in a significant decrease of toxicity[51]. Thus far, there are few examples of successful 5-position modification and 5-NH_2 (SPA), 5-CH_3 (GRE) are the most beneficial for *in vitro* activity[52] (**Table 5**).

Table 5 Comparative *in vitro* antimicrobial activity of GRE and SPA



SPA



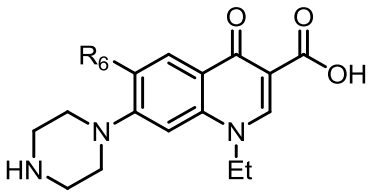
GRE

Compound	*MIC$_{90}$, μM*[a]		
	Gram (–)		*Gram (+)*
	E. coli	*P. aeruginosa*	*S. aureus*
GRE	0.17	22.3	0.33
SPA	0.15	10.2	0.31

Data from ref [52] [a]Compounds were tested against collection of clinical isolates; MIC_{90} is MIC for 90% of strains tested.

Original quinolones were often unsubstituted at C-6 position. The main finding of the pioneering work of *Koga et al.*[5] illustrated a very clear superiority of NOR, a derivative bearing a fluorine atom at the 6 position over its non-fluorinated analog (**Table 6**).

Table 6 *In vitro* antimicrobial activity of C-6 fluorinated vs non-fluorinated NOR

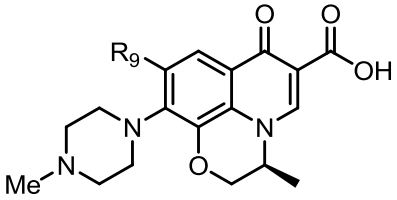


R_6	*MIC (μM)*		
	Gram (–)		*Gram (+)*
	E. coli[a]	*P. aeruginosa*[b]	*S. aureus*[c]
F (NOR)	0.16	1.22	1.22
H	2.59	10.4	41.5

Data from ref [5] [a]*E. coli* NIHJ JC-2 strain [b]*P. aeruginosa* V-1 strain [c]*S. aureus* 209P strain

Strong increase of activity due to fluorine atom at C-9 has also been demonstrated among LEV derivatives[53] (**Table 7**).

Table 7 *In vitro* antimicrobial activity of C-9 fluorinated vs non-fluorinated LEV



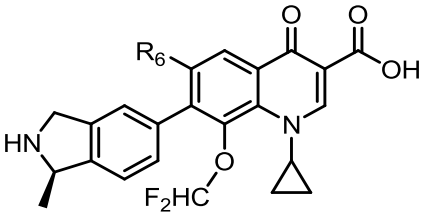
R_9	*MIC (μM)*		
	Gram (–)		*Gram (+)*
	E. coli[a]	*P. aeruginosa*[b]	*S. aureus*[c]
F (LEV)	0.044	1.	0.33
H	23	>93	12

Data from ref [53] [a]*E. coli* ES142 strain [b] *P. aeruginosa* PS 96 strain [c]*S. aureus* MI246 strain

It has become almost a dogma that a fluorine atom at C-6 position (or C-9 position on benzoxazine skeleton) is essential for antibacterial activity. However, several studies have shown that positive effect of a C-6 fluorine atom is diminished when the molecule contains other helpful substituents. The development of the first marketed drug without fluorine atom at C-6 position – Garenoxacin[54] (GAR, R_6 = H, **Table 8**) resulted from a number of analogs by fine

tuning of other substituents in order to minimize toxicity while maintaining high levels of potency.

Table 8 Antimicrobial activity and IC_{50} of GAR and its fluorinated analog

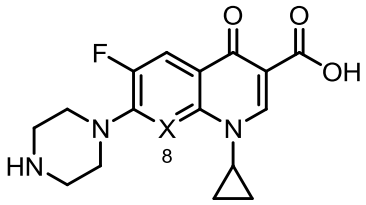


R_6	$IC_{50}(\mu M)$		$MIC\ (\mu M)$	
	E. coli Gyrase[a]	*S. aureus topo IV*[b]	*Gram (–) E. coli*[c]	*Gram (+) S. aureus*[d]
H (GAR)	0.40	5.14	0.14	0.016
F	0.36	10.4	0.28	0.016

Data from ref [54] [a]Corresponds to 50% inhibition of gyrase supercoiling activity [b]Corresponds to 50% inhibition of topoisomerase IV decatenation activity [c]*E. coli* A29179 strain: genotype GyrA Ser83Leu [d]*S. aureus* MR A27223 strain: genotype homogeneous MR

It has long been known that the substituent at C-8 position controls *in vivo* efficacy and affects the antibacterial spectrum. The 8-position substituent can improve anaerobic activity, modulate physicochemical properties and adverse effects. The most commonly studied groups at C-8 position were X= C-H, C-F, C-Cl and N, with the following order of *in vitro* antibacterial potency: C-Cl ≈ C-F > C-H > N[55] (**Table 9**). While 8-F and 8-Cl substituents showed improved activity, these groups were associated with a serious adverse effect of phototoxicity[56]

Table 9 *In vitro* activities of C-8 modified quinolones



X	*Gyrase-drug induced cleavage[a] E. coli H560 IC_{50}(μM)*	*MIC (μM)*	
		Gram (–) E. coli[b]	*Gram(+) S. aureus[c]*
N	3.0	0.15	4.8
C-H (CIP)	1.5	0.15	9.4
C-F	1.44	0.29	1.1
C-Cl	1.4	0.068	0.27

Data from ref [55] [a]Minimum concentration of drug needed to produce linear DNA at an intensity relative to oxolinic acid at 10 μg/mL [b]*E. coli* Vogel strain [c]*S. aureus* H228 strain

The 8-OMe group was found to improve the potency against *S. pneumoniae* resistant strains with alterations in target enzymes[57] and to slower the development of quinolone resistance[58]. In contrast to 8-F derivatives, 8-methoxy quinolones are photostable and do not cause phototoxicity[58]. The 8-OMe quinolone MOX (**Tableau 1**) is characterized by a safety profile comparable to that of older monofluorinated quinolones[59].

1.4.2 Position C-7

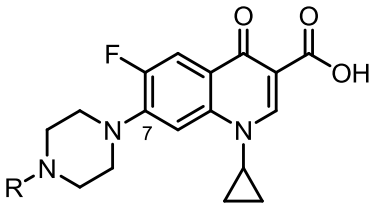
The C-7 position is one of the most widely explored among all other positions. From numerous studies it was deduced that this position determines the antibacterial spectrum, may influence target preference and is tightly related to pharmacokinetics. In the foregoing section we will discuss separately “dimeric” and “monomeric” C-7 modified compounds.

1.4.2.1 Monomers

Many thousands of analogs have been prepared employing various substituents at this promising position, leading to conclusion that a cyclic system containing a secondary or tertiary amine moiety is preferred[2].

After the discovery of NOR and PEF the piperazinyl moiety or its N-methyl analogue became a standard feature among the quinolones. Unsubstituted piperazine ring (as in NOR, ENO and CIP) confer potency against Gram-negative bacteria, while addition of methyl groups can improve both oral absorption[6b] and anti-Gram-positive activity. However, the improved activity against Gram-positive bacteria can sometimes be at the expense of activity against *P. aeruginosa*[60] (**Table 10**).

Table 10 Influence of C-7 piperazine methylation on antibacterial spectrum

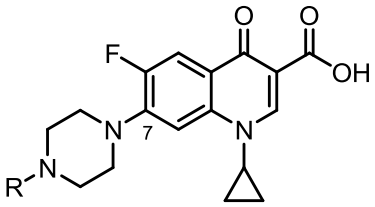


R	*MIC (μM)*		
	Gram (–)		*Gram (+)*
	E. coli[a]	*P. aeruginosa*[b]	*S. aureus*[c]
H (CIP)	<0.05	0.18	1.51
CH_3	<0.05	1.45	0.36

Data from ref [60] [a]*E. coli* ATCC25922 strain [b]*P. aeruginosa* ATC1C 9027 strain [c]*S. aureus* ATCC29213 strain

These changes in antimicrobial spectrum may be related to the alteration of physicochemical properties of target compounds. As shown in section 1.3.4, more lipophilic compounds exhibit increased penetration into Gram-positive organisms while lowering the porin-mediated influx into Gram-negative bacteria. Indeed, it was observed that lipophilic N-methyl and N-ethyl CIP derivatives are equipotent to CIP against *S. aureus*, while only N-methylated analog displays MIC values toward *E. coli* comparable to that of CIP[61] (**Table 11**). In addition, authors demonstrated that increased lipophilicity had direct positive impact on intestinal absorption rates of compounds[62].

Table 11 Comparative antimicrobial activity of N-alkyl CIP analogs

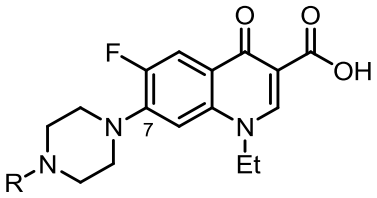


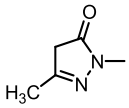
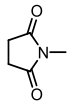
R	*LogP*[a]	*MIC (μM)*	
		Gram (–) E. coli[b]	*Gram (+) S. aureus*[c]
H (CIP)	-1.12	0.076	0.76
Me	0.27	0.073	0.73
Et	0.37	0.14	0.70
n-Pr	1.05	0.27	1.34
n-Bu	1.48	0.51	1.29

Data from ref [62a] [a]P is the bulk phase partition coefficient determined between *n*-octanol and 0.066 M Sörensen phosphate buffer, pH 7.00 [b]*E.coli* ATCC25922 strain [c]*S. aureus* ATCC29213 strain

Recently, *Abuo-Rahma et al.*[63] reported the synthesis, physicochemical parameters and antibacterial activity for a series of N-4-piperazinyl modified NOR (**Table 12**). The authors showed that the succinimide Mannich base and the N-formyl analogue revealed a better activity than NOR in all the tested strains. Interestingly, strong correlation was established between logMIC and LogP values for *P. aeruginosa* ($r^2 = 0.97$), while less evident dependence was found for microorganisms such as *S. aureus* and *E. coli* ($r^2 = 0.67$, 0.62 respectively). These results were explained by the fact that the penetration into bacterial cell is not the only determining factor which can affect activity. Target affinity is another factor which can influence *in vitro* MIC. The difference in the steric bulk at the C-7 substitution may play an important role in the accomodation of these molecules in the active site of enzyme. Another set of correlations between molecular weight and activity were obtained, which are consistent with assumption of porin-mediated quinolone penetration into Gram-negative organisms and passive diffusion through membrane at Gram-positives.

Table 12 MICs of NOR derivatives: Mannich bases

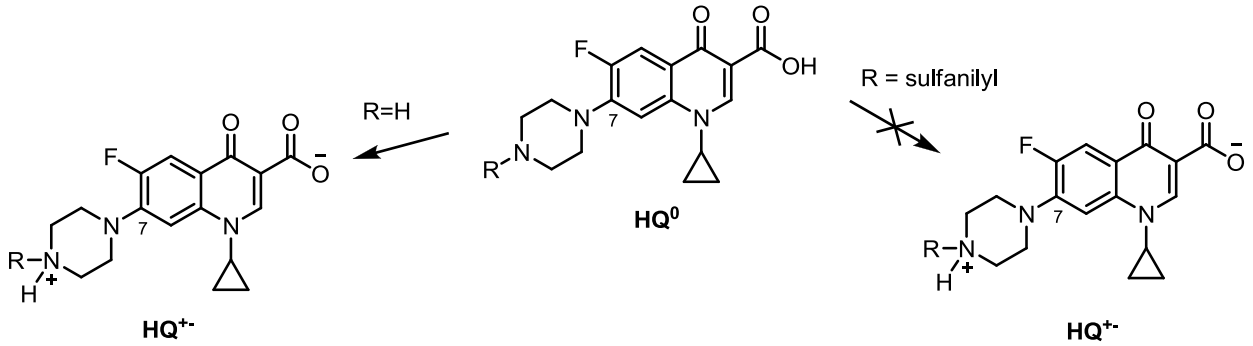


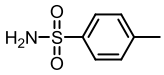
R	*LogP*[a]	M_w	*MIC (μM)*		
			S. aureus[b]	*E. coli*[b]	*P. aeruginosa*[b]
H (NOR)	1.49	319	200	44	28
	0.55	347	<2.9	32	17
	1.16	429	23	<2.4	21
	0.43	430	9.3	<2.3	12

Data from ref [63] [a]Calculated by ACD/labs (TM) software [b]Bacterial strains were supplied from department of Microbiology, Faculty of Pharmacy, El-Minia University.

Studies have shown that substitution at C-7 position could also alter the distribution between charged and uncharged quinolones species in solution. *Alovero et al.*[64] reported the synthesis and *in vitro* antibacterial activity of N-piperazinyl sulfanilyl substituted FQs. These derivatives exhibited more favorable kinetics of access to *S. aureus* cells than do unmodified quinolones. It was postulated that this modification at piperazine ring greatly reduces basicity of distal nitrogen, therefore leaving only one ionizable carboxyl group in the biological pH range. In comparison with zwitterionic parent quinolones (R = H), N-sulfanilyl derivatives (R = H_2N-SO_2-C_6H_4-) have higher fraction of non-ionizated species (**HQ^0**) which are the one responsible for uptake in Gram-positive organisms (**Table 13**). This factor appears to be of primary importance in determining their high *in vitro* activity against *S. aureus*.

Table 13 *In vitro* antimicrobial activity of N-sulfanilyl CIP derivatives



R	*MIC (μM)*	
	Gram (–) *E. coli*[a]	*Gram (+)* *S. aureus*[b]
H (CIP)	0.75	0.09
	0.26	4.1

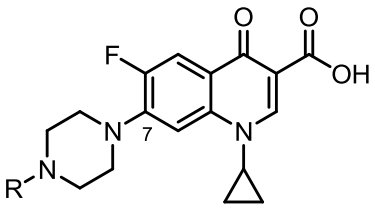
Data from ref [64b] [a]*E. coli* ATCC 25922 strain [b]*S. aureus* ATCC29213 strain

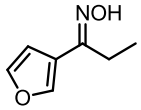
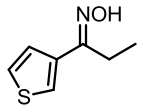
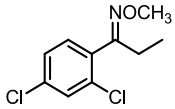
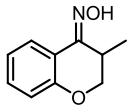
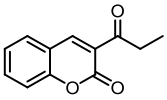
Another important result is that modifications at C-7 position determine not only the potency but also the target enzyme preference. The authors established that unlike unsubstituted CIP which targets topoisomerase IV in *S. pneumoniae*, N-sufanilyl CIP derivatives demonstrated a target preference for DNA gyrase. Moreover, N-sulfanilyl CIP selected first-step mutant with alterations at QRDR of DNA gyrase. These genetic studies revealed that 4-aminobenzenesulfonamide group attached to the distal nitrogen at piperazine moiety provokes target shift in *S. pneumoniae* from topoisomerase IV to DNA gyrase and increases activity *in vitro*[65]. However, it is not clear whether the target shift is related with improved potency or not.

It is worth to mention N-piperazinyl modified CIP derivatives synthesized by *Foroumadi et al.* (**Table 14**). The development started from derivatives containing a N-[2-(furan-3-yl)ethyl][66] and N-[2-(thiophen-3-yl)ethyl[67] residues. These compounds exhibited improved activity against Gram-positive *S. aureus*, including methicillin-resistant strains (MRSA) with respect to CIP. Generally, these agents were significantly less active than parent quinolone towards Gram-negative *P. aeruginosa* and *E. coli* (**entry 2, 3 Table 14**). Further exploration yielded a series of compounds with attached phenyl moiety[68]. Tuning the substitution pattern at phenyl ring allowed to greatly increase their potency against Gram-negatives, while maintaining activity generally comparable to that of parent quinolone against Gram-positives *S. aureus* and MRSA (**entry 4**, **Table 14**). More recently, the authors created conformationally-constrained

analogs[69] (**entry 5**, **Table 14**). This approach lead to coumarine based compound[70] that exhibited improved potency against Gram-positive *S. aureus* and *MRSA*. It is noteworthy to mention that this derivative was more active against *E. coli* and *P. aeruginosa* in comparison with CIP which exhibits one of the lowest MIC towards Gram-negatives (**entry 6, Table 14**).

Table 14 Antibacterial activity of various *N*-substituted piperazinylquinolones synthetized by *Foroumadi et al.*



N	*R*	*MIC (μM)*					*Ref*
		Gram (–)		*Gram (+)*			
		E. coli[a]	*P. aeruginosa*[b]	*S. aureus*[c]	*MRSA I*[d]	*MRSA II*[d]	
1	H (CIP)	0.04	1.2	0.57	1.2	1.2	66
2		0.42	28	0.22	0.42	0.42	66
3		0.83	>212	0.21	0.40	0.40	67
4		0.046	2.9	0.71	0.71	0.71	68
5		1.6	102	0.1	0.2	-	69
6		0.025	0.75	0.37	0.75	0.75	70

[a]*E. coli* ATCC 8739 strain [b]*P. aeruginosa* ATCC 9027 strain [c]*S .aureus* ATCC 6538p strain [d]MRSA I and II: methicillin-resistant *S. aureus* (clinical isolates I and II)

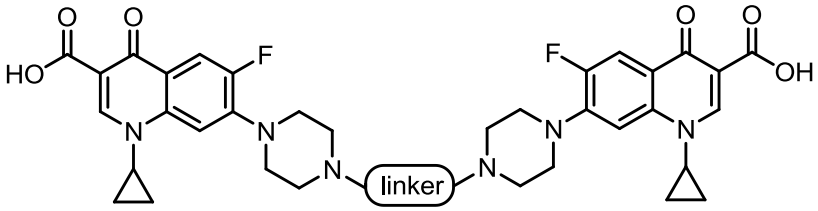
All these reports showed that C-7 position possesses unique features, like great tolerance for modifications coupled with high influence on antibacterial potency, spectrum, target preference and physiochemical properties. Cyclic amines are preferred substituents at this position, with piperazine and aminopyrrolidine being the most popular. Attachment of different

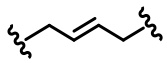
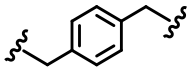
groups to the distant 4-nitrogen atom at piperazine moiety, generally increases the potency against Gram-positives bacteria, including MRSA and lowers activity towards Gram-negatives. These results could be related to the fact that increased steric bulk and lipophilicity are better tolerated in Gram-positive organisms with a single cellular membrane. In contrast, substitutions at piperazine moiety that increase lipophilicity and steric bulk, generally decrease the potency towards Gram-negatives. However, no single property of C-7 modification determines activity. The combination of different factors, like structural features, LogP, molecular weight governs the degree and the spectrum of activity.

1.4.2.2 "Dimers"

Since the first insights into mechanism of inhibition by quinolones it was proposed that there is more than one molecule present in the quinolone-enzyme complex. In 1989, *Shen et al.*[71] reported for the first time, the coupling of two quinolone pharmacophores in a single molecule. He showed that among the synthesized N-1 linked NOR "dimers", the derivative containing 4 methylene units, was the most potent against *E. coli* gyrase (with IC_{50} value roughly equal to that of NOR). Further study of *Kerns et al.*[72] identified piperazinyl-linked CIP "dimers" that displayed equivalent or lowered MIC values compared with those of parent FQ against *S. pneumoniae* and *E. coli*. However, raised MIC values were observed for "dimers" in comparison with CIP against *P. aeruginosa* (**Table 15**). The most potent linkers were *trans*-butenyl and *para*-xylenyl and corresponding "dimers" were tested against wild-type isolate and drug-resistant strains of *S. aureus.*

Table 15 Piperazinyl-linked CIP "dimers": spectrum of antibacterial activity

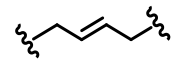
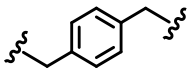


Linker	*MIC (μM)*		
	Gram (–)		*Gram (+)*
	E. coli[a]	*P. aeruginosa*[b]	*S. pneumoniae*[c]
 *trans*-butenyl	0.17	>22	0.17
 *para*-xylenyl	0.33	>21	1.3
— (CIP)	<0.9	1.5	0.75

Data from ref [72-73] [a]*E. coli* R973 strain [b]*P. aeruginosa* 27853 strain [c]*S. pneumoniae* 49616 strain

These CIP-based "dimers" displayed up to 10-fold increased activity against wild-type *S. aureus* and methicillin-resistant strains of *S. aureus*[73] (**Table 16**). More interestingly, a 50-fold increased potency was observed against resistant mutant possessing both *NorA* efflux-mediated resistance and a topoisomerase IV mutation. The authors also demonstrated that dimerization affected the target preference in *S. aureus*. In fact, the target of "dimer" in *S. aureus* is primarily DNA gyrase, whereas topoisomerase IV is the primary target of CIP[74]. This could explain increased potency against topoisomerase IV mutant strain. The authors also reported similar switch in target preference in *S. pneumoniae*[75] (**Table 16**).

Table 16 CIP **"**dimers": antibacterial activity against wild-type and resistant strains of *S. aureus*

Linker	*MIC (μM)*			
	Strains of S. aureus			
	S. aureus 1199[a]	*S. aureus 1199-3*[b]	*S. aureus 1199B*[c]	*MRSA 494*[d]
 *trans*-butenyl	0.04	0.08	0.04	0.17
 *para*-xylenyl	0.16	0.16	0.16	0.16
— (CIP)	0.38	3.0	24	1.5

Data from ref [73] [a]SA 1199, wild-type isolate; [b]SA 1199-3, mutant with *norA* gene overexpression (efflux-mediated resistance) and no gyrase or topo IV mutations; [c]SA-1199B, constitutively overexpresses *norA* and harbors topo IV mutation (A116E), known to correlate with raised FQ MICs; [d]MRSA 494, methicillin resistant *S. aureus* isolate.

These reports demonstrated that some piperazinyl linked "dimeric" compounds displayed:

- Increased activity against Gram-positive *S. aureus* and *S. pneumoniae*
- Switch in target preference from topo IV to DNA gyrase (*S.aureus and S. pneumoniae)*
- Increased potency against various *S. aureus* resistant strains, including MRSA

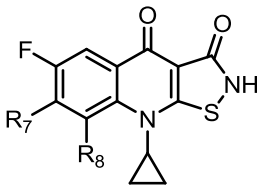
All these features make "dimeric" quinolones interesting subject of further research.

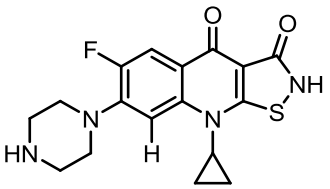
1.4.3 Position C-3

The C-3 position is one of the less explored in the quinolone core, probably due to initial modifications which were usually unsatisfactory[48b]. Positions 3 and 4 must be a carboxyl group and oxygen atom respectively, because quinolone binding at ternary complex drug-DNA-enzyme occurs primarily at these positions. *Chu et al.*[76] have shown that bioisosteric substitution of the carboxylic acid at C-3 is generally detrimental to the antibacterial activity of quinolones. However, isothiazoloquinolones (ITQs) represent one of the few examples of successful replacement of the 3-carboxylic acid of FQs (with a ring-fused isothiazolone) without

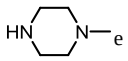
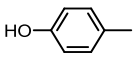
compromising antibacterial activity. The subsequent work of *Chu et al*[77] initially investigated compound A-62824 (**Table 17**), in which the 3-carboxylic acid group of CIP is replaced by a fused isothiazolo ring and demonstrated that A-62824 was more potent (4 to 10 times) *in vitro* than CIP and possessed enhanced activity against DNA gyrase. The isothiazolo ring system in A-62824 possesses an aromatic character and the nitrogen proton which is very acidic, can be considered to mimic carboxylic acid. The authors also suggested that the planarity between the 4-oxo group and the enolized isothiazolo group must be important for the DNA gyrase binding. Unfortunately, these molecules also inhibited mammalian topoisomerase II[78] and caused mammalian cytotoxicity. Consequently, ITQs were underexplored until 2006 when *Wiles et al.* reported that several ITQs bearing functionalized with phenyl[79] or heteroaromatic groups[80] at the C-7 position, displayed reduced cytotoxicity in Hep2 human laryngeal carcinoma cell line and increased activity against a multi-drug-resistant *S. aureus* strain when compared with A-62824, CIP and MOX (**Table 17**).

Table 17 Antibacterial and cytotoxic activities of ITQs





A-62824

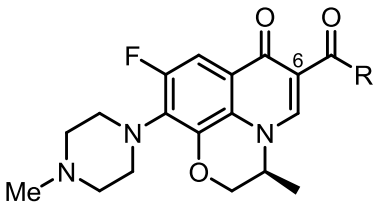
R_7	R_8	*MIC (μM)*			CC_{50} *(μM)*[d]
		Gram (–)	*Gram (+)*		
		E. coli[a]	*S. aureus*[b]	*MRSA*[c]	
	H	0.01	0.14	11	7
	H	0.08	0.01	0.34	8
	H	0.16	0.01	1.36	36
	OMe	–	0.005	0.30	>100
CIP		0.06	0.76	96.68	>100

Data from ref [79-81] [a]*E. coli* ATCC 25922 strain [b]*S. aureus* ATCC 29213 strain [c]methicillin-resistant, quinolone-resistant, vancomycin intermediate-resistant *S. aureus* ATCC 700699 strain [d]Cytotoxic concentration: Concentration that reduces the proliferation of Hep2 human laryngeal carcinoma cells by 50% after 72h [e]A-62824

Further modification of the ITQ nucleus at C-8 position[81] provided the 8-methoxy derivative with the most attractive *in vitro* biological profile (**Table 17**). ITQs displayed broad-spectrum activities especially against Gram-positive pathogens, including several antibiotic-resistant organisms, such as MRSA, vancomycin-resistant *S. aureus* (VRSA) and quinolone-resistant strains[82]. Moreover, ITQs were proved to interact with both DNA gyrase and topoisomerase IV – so called dual-targeting mode of inhibition, with the preference for DNA gyrase[83]. Genetic studies suggested the potential utility of heteroaryl isothiazolones in combating infections caused by *S. aureus*, including multidrug-resistant MRSA.

Another interesting approach is represented by the introduction of amide moiety as a mimic of carboxyl group. *Patel et al.*[84] described the synthesis and antimicrobial activity of CIP carboxamides, whereas LEV carboxamides were evaluated by *Sultana et al.*[85]. Significant enhancements of potency towards Gram-positive organisms were achieved in comparison with unmodified precursor (**Table 18**)

Table 18 Zone of inhibition of LEV and its C-6 carboxamide derivative



R	*Zone of inhibition, mm*[a]			
	Gram (–)		*Gram (+)*	
	E. coli	*P. aeruginosa*	*S. aureus*	*S. pneumoniae*
OH (LEV)	16	14	12	16
NH-C_6H_5	15	14	16	19

Data from ref [85] [a]Diameter of inhibited zone proportional to the bacterial susceptibility to the antimicrobial present in the disk; measured at 10 ppm concentration. The bacterial strains are not indicated.

In this section, we have shown studies demonstrated that in spite of the low tolerance for a structural modification an efficient mimic of carboxylate group with acidic moiety could be introduced at C-3 position. This would increase *in vitro* potency and broader the spectrum of activity. Varying substituents at this position could also control the target preference.

2. Quinolones as antimycobacterial agents

2.1 Introduction

Tuberculosis (TB) is one of the most common infectious diseases known to humans. About 32% of the world's population (1.9 billion people) is infected with TB. Every year, approximately 9.4 million of the infected people develop active TB and almost 1.8 million of these infected people die from the disease, a life lost due to TB every 15 s. The first-line drugs currently used for the treatment of TB, an infection caused by *M. tuberculosis*, are isoniazid, ethambutol, pyrazinamide and rifampicin (**Figure 8**)[86]. However, TB is still a challenging worldwide health problem, especially due to the emergence of multi-drug resistant (MDR) strain of *M. tuberculosis* that is resistant to, at least, the two first-line medications, isoniazid and rifampin. It is estimated that, 5% of the more than 9 million persons who develop TB every year are infected with a MDR strain of TB[87].

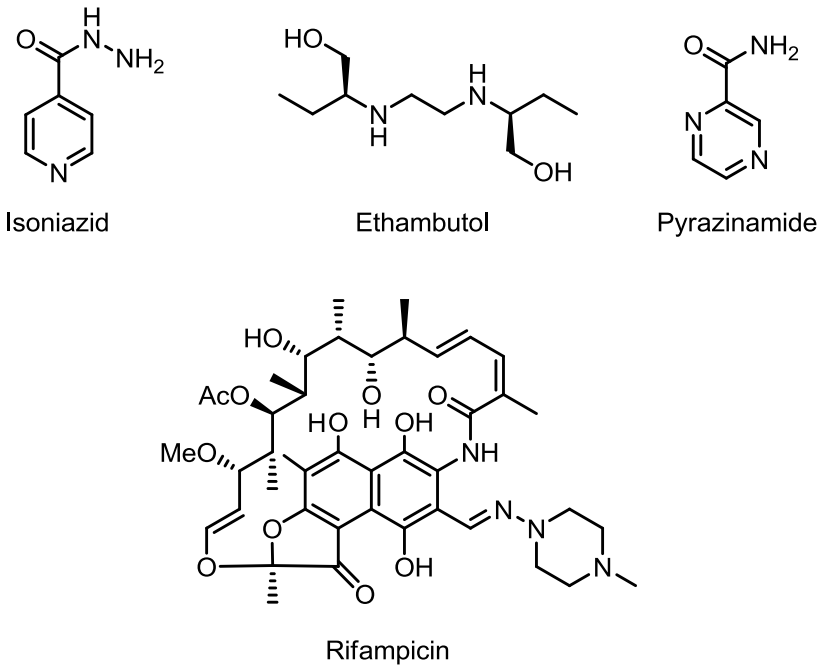


Figure 8 WHO recommended first-line drugs in TB treatment

The anti-TB activity of FQs has been under investigation since the 1980s. Many are active *in vitro* but only a few, including OFL, CIP, SPA, LEV, MOX have been clinically tested[88]. On the basis of these studies CIP, OFL and LEV have been suggested as alternative treatments in TB (second-line therapy). FQs and injectable agents (aminoglycosides such as amikacin, kanamycin and peptide capreomycin, **Figure 9**) are the most effective second-line

anti-TB drugs and the only ones that have bactericidal effect. They are therefore recommended in the initial phase of any MDR-TB treatment regimen.

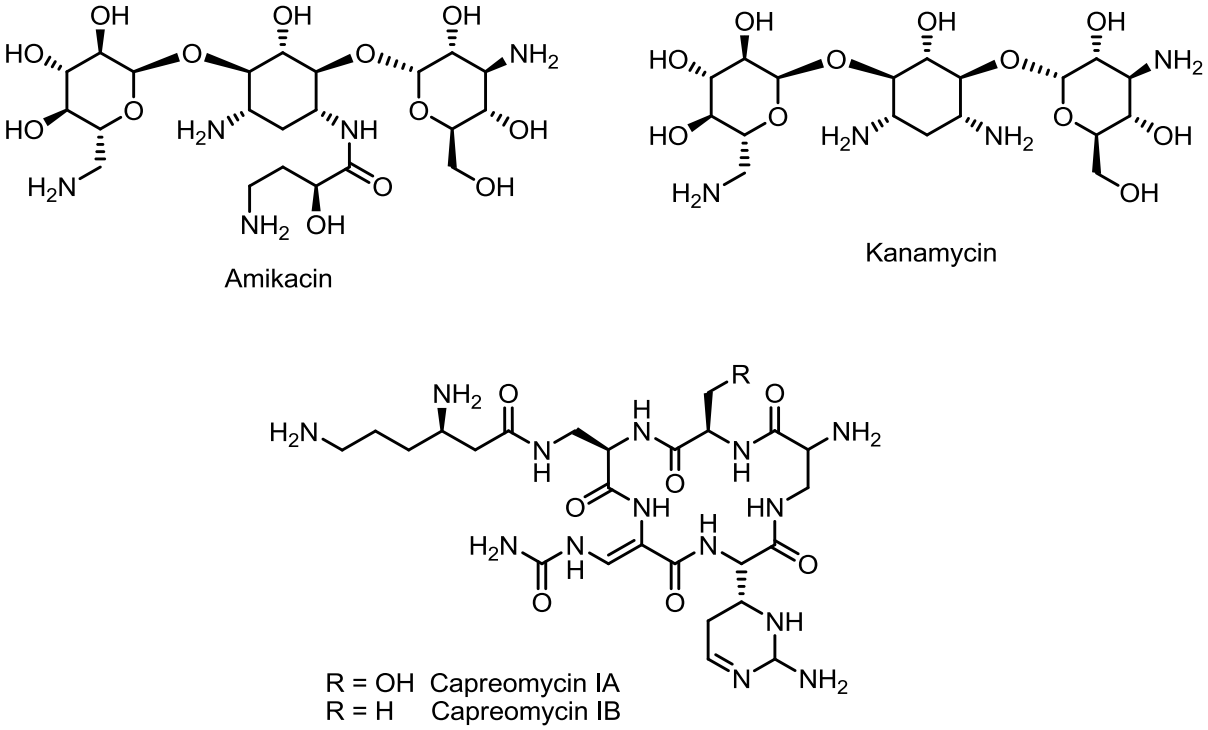


Figure 9 Examples of second-line antiTB drugs

In 2006, extensively drug-resistant (XDR) tuberculosis emerged, which is basically MDR-TB that is additionally resistant to at least one of the FQs and one of the three injectable second-line drugs[89]. Combining data from 46 countries that have reported continuous surveillance of drug resistance showed that 5.4% of MDR-TB cases were found to have XDR-TB. Furthermore, the association of TB and HIV infections has caused an urgent need in search of alternative chemotherapeutics for *M. tuberculosis* infection.

The 13th annual report on global control of TB published by World Health Organization (WHO) in 2009 recommended LEV, OFL and MOX as a second-line drug in treating MDR-TB and drug-susceptible TB[90].

2.2 Mechanism of action and resistance

2.2.1 Quinolone target in M. tuberculosis

As previously described in section 1, the bacterial targets of quinolones are type II topoisomerases (DNA gyrase and topoisomerase IV) which grant quinolone antibiotics with dual-inhibition mode, lower rates of resistance emerging and high potency. In 1998 the full genome sequence of *M. tuberculosis* was revealed and two genes *gyrA* and *gyrB* were identified to encode A and B subunits of DNA gyrase[91]. However, contrary to "common" bacteria there is no evidence of the *parC* and *parE* genes for topoisomerase IV in the genome of *M. tuberculosis*. DNA gyrase is the only type II topoisomerase present in this organism and hence is the only target for FQ action. Because topoisomerase IV is necessary for the decatenation of daughter chromosomes during DNA replication; functions of the single *M. tuberculosis* type II topoisomerase were explored by *Aubry et al.*[92] The intermolecular passage activities of purified DNA gyrase from *M. tuberculosis* was demonstrated: decatenation activity of this enzyme was 30-fold higher than that of *E. coli* DNA gyrase, but was lower than that of topoisomerase IV from *S. pneumoniae*. Overall, the type II topoisomerase from *M. tuberculosis* exhibits classical supercoiling activity of DNA gyrase while having additional decatenation topo IV like activity. The absence of dual inhibition together with unique topoisomerase protein sequence marks out *M. tuberculosis* among "common" Gram-positive/negative bacteria.

2.2.2 Penetration into Mycobacterium cell

Bacterial topoisomerases are intracellular targets and quinolones have to pass through the cell wall to get into the intracellular space. *Mycobacterium* species share a characteristic cell wall, thicker than in many other Gram-positive and Gram-negative bacteria, which is hydrophobic, waxy and rich in mycolic acids/mycolates[93]. Mycobacteria possess, in addition to phospholipid bilayer and peptidoglycan, the hydrophobic layer of long chain (C_{70}-C_{80}) fatty acids known as mycolic acids. They are held together with peptidoglycan layer by the polysaccharide, arabinogalactan (**Figure 10**).

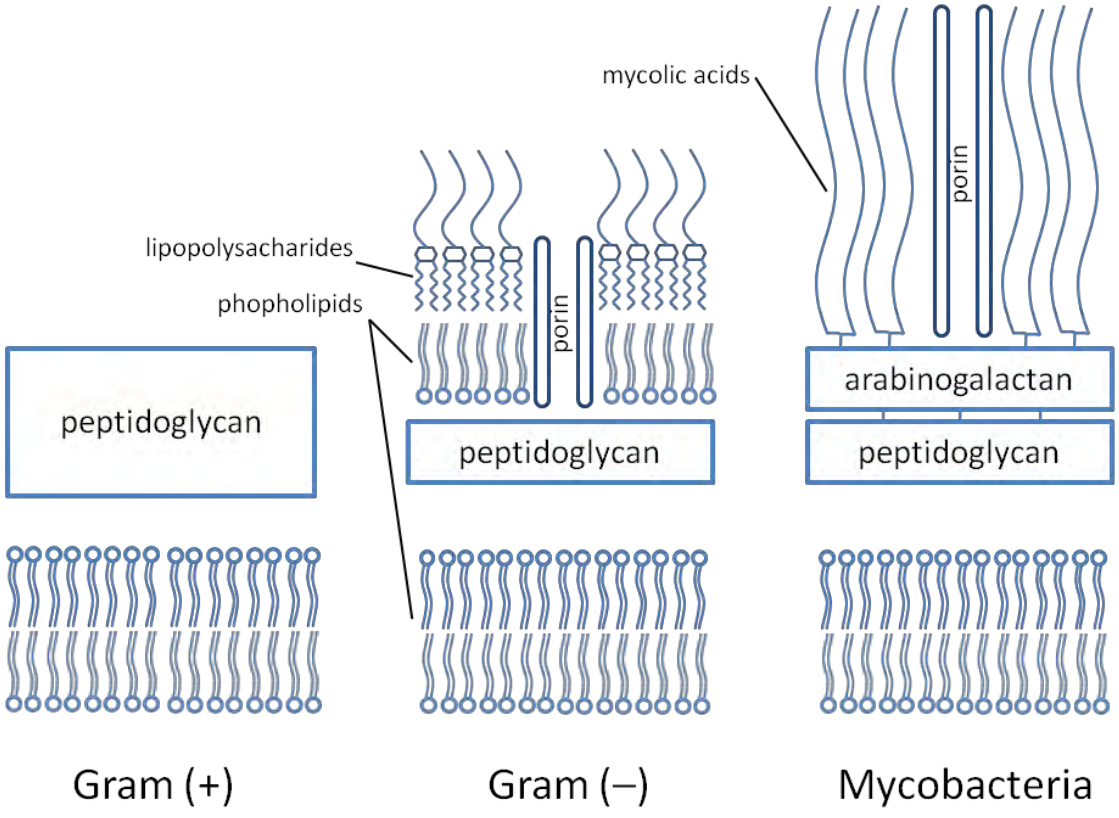


Figure 10 Structure of *Mycobacterium* cell wall in comparison with Gram-positive and Gram-negative bacteria.

The low permeability of the mycobacterial cell wall, with its unusual structure, is now known to be a major factor in intrinsic antibiotic resistance of *M. tuberculosis*[94]. In addition to the cell wall, there are evidences for the outer capsule existence that creates a barrier for diffusion of foreign molecules into the inner parts of the envelope[95]. It is supposed that more hydrophilic agents cross the cell wall slowly because the mycobacterial porin is expressed in low quantities and is inefficient in allowing the permeation of solutes. More lipophilic agents are presumably slowed down by the lipid bilayer which is of unusually low fluidity and abnormal thickness.

2.2.3 Resistance to quinolones

In 1970s a dramatic decline in the prevalence of TB infections was observed and TB ceased to be a problem in industrialised countries. Unfortunately, TB yet remains one of the most deadly infections primarily due to the emergence of resistance development. As the rate of mutations conferring resistance to multiple drugs is very low, the WHO-recommended standard chemotherapy consists of a combination of four first-line drugs (isoniazid, ethambutol, pyrazinamide and rifampicin), while second-line drugs (FQs, aminoglycosides) are mostly used

in the treatment of MDR-TB. Another approach has been proposed to prevent resistance; the FQ must be administred at doses that produce serum concentrations that exceed the "mutant prevention concentration" (MPC). This drug concentration is capable of inhibiting all spontaneous first-step mutants[96]. However, to date there is no quinolone which possesses such a good pharmacodynamic safety profile and high degree of activity.

Emerging resistance poses a serious problem on the way of the world-wide eradication of TB and there is a constant need for new antimycobacterial agents.

2.2.3.1 Mutations at the target site

The primary source of quinolone resistance in *M. tuberculosis* is mutations at the DNA gyrase. Various studies demonstrated that laboratory-selected FQ resistant mutants of *M. tuberculosis* showed exactly the same changes described in clinical isolates. Significant resistance to FQs can be achieved with a single gyrase mutation, whereas at least two mutations (two mutations in *gyrA*, or mutations in *gyrA* plus *gyrB*) found to be necessary for the highest level of resistance. Mutations encoutered in FQ-resistant *M. tuberculosis* were located in codons equivalent to those in *GyrA* genes of other FQ-resistant bacteria. The most frequently mutated codons were: codon 90 (with a Ala/Val change), codon 91 (Ser/Pro) and codon 94 (with five different amino acid change: Asp/Ala-Gly-His-Tyr or Asn)[97].

2.2.3.2 Efflux mediated resistance

Previously mentioned cell wall barrier alone cannot produce significant level of drug resistance, which requires synergistic contribution from a second factor, such as the efflux pumps. Recent evidence suggests that *Mycobacteria* extrude many drugs via active efflux systems[98] and efflux-mediated FQ resistance has been described in the *Mycobacterium smegmatis (M. smegmatis)*[99]. The LfrA protein gene has been identified in *M. smegmatis*; it was shown to have greater affinity for hydrophilic FQ's (CIP, LEV) than for lipophilic ones (SPA, NAL)[100].

2.2.3.3 DNA mimicking

In 2001 genetic selection for FQ resistance in *M. smegmatis* identified a new type of resistance mechanism mediated by the MfpA protein[101]. The sequence of MfpA revealed it to be a member of the "pentapeptide repeat" family of bacterial proteins, in which every fifth amino

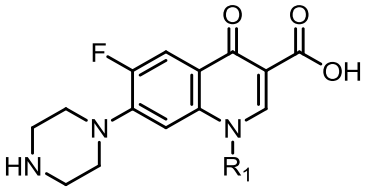
acid is either a leucine or phenylalanine. This protein forms an adduct with gyrase from *M. tuberculosis* and inhibits its activity[102].

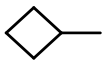
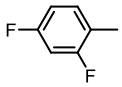
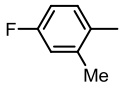
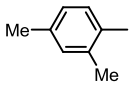
2.3 Structure-activity relationships

2.3.1 General trends

In comparison with thousands of quinolones tested against "common" bacteria, there are less data on structure activity studies for Mycobacteria. Because *M. tuberculosis* divides very slowly (about 15-20 hours for division), 20-30 days are necessary for testing. In addition, analysis requires special protective conditions. For all these reasons, several authors utilized less hazardous and rapidly growing *M. fortuitum* to estimate the quinolone potency against *M. tuberculosis*. Testing results against *M. smegmatis* were often included for comparative purposes. Generally, *M. fortuitum* was more sensitive to the compounds than *M. smegmatis*. *Renau et al.*[103] carried out systematic studies focused on the influence of lipohilicity at N-1 and C-7 positions on quinolone antimycobacterial activity. The evident rationale for these studies was the fact that, unlike "common" bacteria, *M. tuberculosis* possesses a thick and lipophilic cell wall that forms a transport barrier for antibiotics. However, the authors showed that "intrinsic" activity against Gram-positive and/or Gram-negative bacteria is the important factor for antimycobacterial activity, more determining than lipophilicity of N-1 substituent. The results summarized (**Table 19**), demonstrated that compounds with close CLogP values exhibit dramatic differences in activities toward *Mycobacteria*.

Table 19 Comparative effect of N-1 substituents on antibacterial / antimycobacterial activity



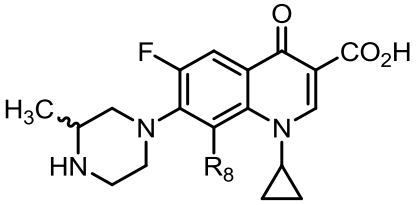
R_1	$CLogP^e$	*MIC (μM)*			
		Bacteria G(–)/G(+)		*Mycobacteria*	
		E. coli[a]	*S. aureus*[b]	*M. fortuitum*[c]	*M. smegmatis*[d]
Et (NOR)	2.21	0.31	9.7	1.6	6.3
 (CIP)	2.04	0.15	2.4	0.18	0.75
i-Pr	2.52	2.4	>9.3	3.0	12
	2.60	1.2	9.0	1.5	5.8
t-Bu	2.92	0.6	2.3	<0.09	0.37
	4.85	0.25	1.0	0.62	1.2
	5.20	0.5	2.0	2.5	10
	5.53	2.0	>7.8	41	81

Data from ref [103] [a]*E. coli* Vogel strain [b]*S. aureus* H-228 strain [c]*M. fortuitum* ATCC6841 strain [d]*M. smegmatis* ATCC19420 [e]A computational model (MedChem Version 3.54, Pomona College, Pomona, CA) was used to determine the theoretical distribution coefficients (clog P) for each compound

In this study, the most active agents against mycobacteria were compounds substituted at N-1 with a *tert*-butyl and cyclopropyl group. CIP was further modified at C-7 piperazine moiety with side chains bearing various alkyl substituents in order to modify the lipophilic character of the heterocycle[103]. Detailed results on the influence of the C-7 substituent on the antimycobacterial activity will be discussed in the corresponding section.

C-8 substituent has influence on the potency against *Mycobacteria.* In the case of *M. smegmatis* the following order was demonstrated for activity against both wild-type strains and resistant mutants: OMe ≈ Cl > Br > F > H[104] (**Table 20**). Moreover, the C-8 methoxy group, when compared to unsubstituted C-8 analog greatly reduced the selection of resistant mutants of *M. tuberculosis*[105].

Table 20 Influence of C-8 position on the activity against *M. smegmatis*



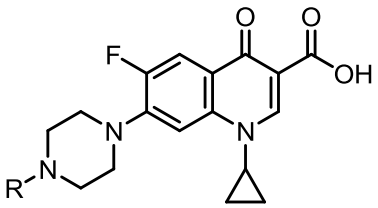
R_8	*M. smegmatis*, MIC_{99} (μM)[a]	
	gyrA[+b]	*gyrA*[c]
H	0.290	8.10
F	0.099	3.30
Br	0.075	2.26
Cl	0.076	1.73
OMe	0.080	1.30

Data from ref [104] [a]The MIC at which 99% of the isolates tested were inhibited [b]*M. smegmatis* wild-type mc^{2}155 strain [c]*M. smegmatis gyrA* (Asp95Gly) quinolone-resistant mutant KD2003

2.3.2 SAR at C-7 position

C-7 position tolerates large structural changes, but an important difference between mycobacteria and other Gram-positive or Gram-negative bacteria was demonstrated. *Haemers et al.*[106] studied the influence of N-substitution of C-7 piperazine on antimycobacterial activity of CIP. Derivatives with longer alkyl chain (and higher lipophilicity) showed higher antimycobacterial activity. However, introduction of bulky phenyl or benzyl substituents lowered the potency in spite of high lipophilicity (**Table 21**).

Table 21 Influence of the C-7 piperazine alkylation on the antimycobacterial activity of CIP

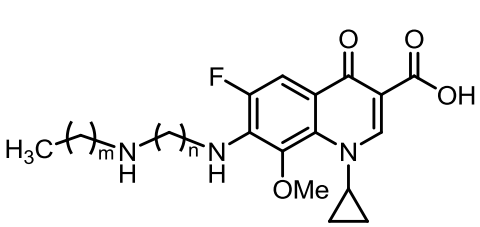


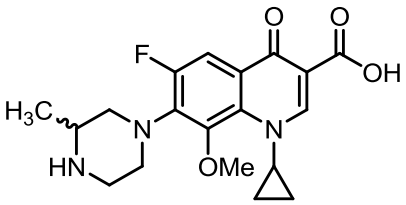
R	*M. tuberculosis, MIC (μM)*	
	MIC_{50}[a]	MIC_{90}[a]
H (CIP)	1.5	3.0
Me	0.36	1.5
Et	0.35	0.70
n-Pr	0.33	0.67
Ph	4.9	9.8
CH_2Ph	2.4	4.8

Data from ref [106] [a]Compounds were tested against collection of *M. tuberculosis* strains; MIC_{50} and MIC_{90} are MIC for 50% and 90% of isolates tested, respectively.

As it was demonstrated that C-8 methoxy FQs are more active against *M. tuberculosis*, *Aldeida et al.*[107] described the anti-TB evaluation of various 8-methoxy-FQ derivatives bearing some mono N-alkylated 1,3-propane diamine substituents at C-7 position. It was observed that, similarly with earlier discussed N-alkyl CIP analogs, in the series of GAT analogs 10 carbon atoms chain length confer maximum potency. In the same time, compounds with shorter or longer chains are less active (**Table 22**).

Table 22 Activity of C-7 modified GAT analogs against *M. tuberculosis* $H_{37}R_v$ (virulent strain)





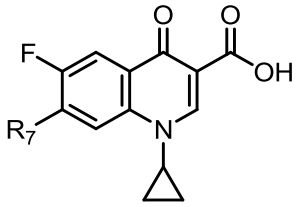
GAT

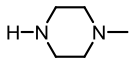
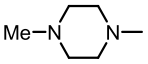
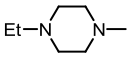
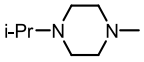
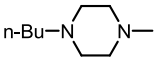
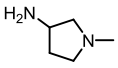
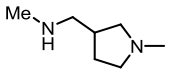
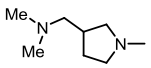
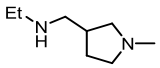
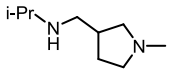
n	*m*	*MIC (μM)*
3	5	115
3	7	2.71
3	9	0.63
3	11	1.20
GAT		0.27

Data from ref [107]

The group of *Renau et al.* conducted systematic studies on the influence of C-7 substituent on the activity against *Mycobacteria*. The authors have demonstrated that N-1 cyclopropyl (and *t*-butyl) quinolones were more active than N-1 2,4-difluorophenyl analogs (**Table 19**)[103]. This report also included over 15 different residues based on piperazine and pyrrolidine side chains at C-7 position. The biological evaluation of this series of compounds revealed that an increase in lipophilicity (via alkyl substitution of piperazine moiety) led to a decrease in antimycobacterial activity. Results from **Table 23** show that in each series the most lipophilic compounds were the less active towards *M. fortuitum*. Comparison between the two series of compounds demonstrated that piperazine-substituted analogs were almost equipotent to pyrrolidine-substituted derivatives.

Table 23 Piperazine vs pyrrolidine side chain at C-7 position: influence on the antimycobacterial activity

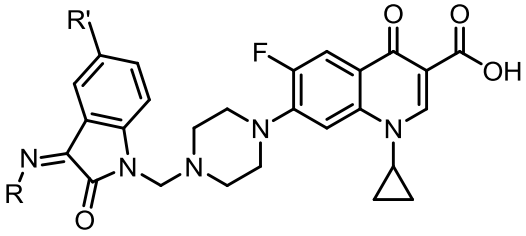


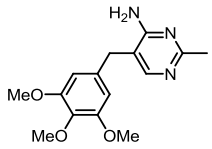
R_7	*CLogP*[a]	*MIC (μM)*	
		M. fortuitum	*M. smegmatis*
 (CIP)	2.04	0.18	0.75
	2.89	0.17	0.72
	3.42	0.17	0.36
	3.73	0.67	0.67
	4.48	2.6	1.3
	1.94	<0.09	0.75
	2.13	0.69	1.4
	2.62	0.35	1.3
	2.66	1.3	1.3
	2.97	0.65	1.3

Data from ref [103] [a]A computational model (MedChem Version 3.54, Pomona College, Pomona, CA) was used to determine the theoretical distribution coefficients (clog P) for each compound

The group of *Sriram et al.*[108] synthesized and evaluated some OFL and CIP derivatives modified at C-7 position. The authors first explored the *in vitro* antimycobacterial activity of some Mannich bases of CIP (**Table 24**) and demonstrated that simply increasing the lipophilic character of the C-7 substituent (as seen for clogP values) increased the activity.

Table 24 Mannich bases of CIP and their antimycobacterial activity

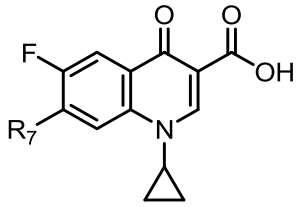


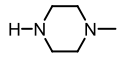
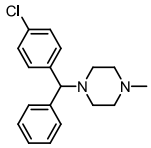
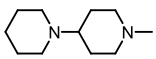
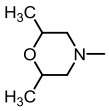
R	*R'*	*CLogP*[a]	*% Inhibition at 6.25 μg/mL*[b]	*MIC (μM)*[b]
$NHCONH_2$	Br	2.04	99	1.98
$NHCSNH_2$	Br	1.66	100	1.88
	F	5.29	100	1.00
CIP		0.01	95	6.04
Ethambutol		–	98	9.22

Data from ref [108] [a]Calculated using online service www.logp.com [b]*M. tuberculosis* $H_{37}Rv$ strain

To investigate this observation in more depth, the authors prepared and evaluated a series of C-7 substituted CIP analogs (**Table 25**)[109]. Biological evaluation showed that compounds with high bulky substituent at C-7 position could display high potency towards *M. tuberculosis*. In addition, the most active derivatives were evaluated against MDR strains of *M. tuberculosis* showing low values of MICs. A comparison of substitution pattern at C-7 position demonstrated the following order of activity: piperidine > piperazine > morpholine.

Table 25 Influence of bulk and liphophilicity at C-7 position on *in vitro* antimycobacterial activity

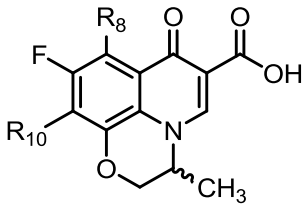


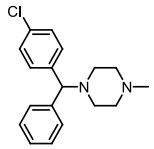
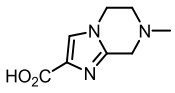
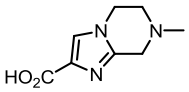
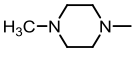
R_7	*MIC* (μM)	
	M. tuberculosis[a]	*MDR-TB*[b]
 (CIP)	6.04	–
	0.17	0.17
	0.94	0.94
	1.08	2.16

Data from ref [109] [a]*M. tuberculosis H37Rv* strain [b]MDR-TB clinical isolate from Tuberculosis Research Center, Chennai, India

The authors recently described a series of OFL analogs bearing various substituents at C-8 and C-10 positions on the benzoxazine moiety (**Table 26**)[110]. The results demonstrated the positive impact of C-8 nitro group on antimycobacterial activity with the following order of activity NO_2 > H > NH_2. Concerning the substitution pattern at C-10 position the authors have found the following trend in antimycobacterial activity: piperidine > morpholine > piperazine; this is quite similar to that obtained for CIP derivatives.

Table 26 Influence of C-8 and C-10 substitution on antimycobacterial activity of OFL derivatives



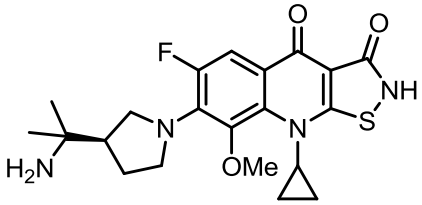
R_8	R_{10}	MIC_{99} (μM)	
		M. tuberculosis[a]	*MDR-TB*
NO_2		2.63	2.63
H		0.91	0.44
NO_2		0.19	0.09
H	 (OFL)	2.16	34.6

Data from ref [110] [a]*M. tuberculosis H37Rv* strain

2.3.3 SAR at C-3 position

Similar to antibacterial potency C-3 position have low tolerance for modification and carboxyl group is generally kept to render high levels of antimycobacterial activity. However, some ITQs that have been already mentioned as potent antibacterials showed noticeable activity against *Mycobacteria*. The most potent compound among them is ACH-702[111] (**Table 27**), which exhibited MICs towards the clinical isolates of *M. tuberculosis* superior or equal to that of marketed quinolones.

Table 27 Chemical structure of ACH-702 and its antimycobacterial activity



Compouund	*M. tuberculosis (31)*[a] *(μM)*	
	MIC_{50}	MIC_{90}
ACH-702	0.14	0.28
MOX	0.30	0.62

Data from ref [111] [a]*M. tuberculosis H37Rv* strain (number of clinical isolates)

In summary, we can conclude that *Mycobacteria* species represent a specific target for FQs with different SAR trends established for other bacteria. Lipophilicity of the antibacterial agent was shown to play an important role but it is not the only governing factor in determining the potency towards *Mycobacteria*. Intrinsic antibacterial activity was also necessary to achieve high levels of antimycobacterial activity. Recent studies have shown that FQs have a full potential of becoming a safe and reliable tool for the treatment of classic and resistant TB.

3. Quinolones as antiproliferative agents

3.1 Introduction

We have described (section 1.3) the quinolone's mechanism of antibacterial action, which comprises inhibition of bacterial type II DNA topoisomerase enzymes (gyrase and topoisomerase IV)[112]. In order to manage DNA, eukaryotic cells have also evolved a topoisomerase II. Eukaryotic type II topoisomerase cannot introduce supercoils; it can only relax them. As a result of its catalytic function, topoisomerase II is necessary for the viability of all organisms from unicellular bacteria to humans and is the target for some of the most active anticancer agents used for the treatment of human malignancies[113].

One of the first reports of possible anticancer effect of quinolones came in the middle of 80's. It was found that antibacterial quinolones such as CIP, NOR and OFL display only slight inhibitory activity against calf thymus topoisomerase II[114]. Meanwhile, to improve the quinolone activity toward Gram-positive bacteria, a series of C-7 aryl derivatives were explored and some of them, bearing 4-pyridinyl substituent, enhanced topoisomerase II-mediated DNA damage and turned out to be too toxic for the use as antibiotic[115]. These early findings provided lead compounds for further exploration of potential antitumor quinolones with different biological targets[78, 116]. As a result of numerous efforts SNS-595 (Voreloxin®)[117] and CX-3543 (Quarfloxin®)[118] were discovered and entered phase II clinical trials on patients. However, currently there is no quinolone-based anticancer drug on the market.

3.2 Eukaryotic quinolone targets

3.2.1 Topoisomerase II

First exploited target in eukaryotic species was topoisomerase II, an enzyme responsible for relaxing superhelical tension in DNA strands during transcription, replication and recombination. Eukaryotic topoisomerase II have been first isolated from lower eukaryotes such as yeast and *Drosophila*; these species have only a single isoform of the enzyme. On the other hand, studies have confirmed the existence of two closely related topoisomerase II isoforms in higher eucaryotes (vertebrate species), termed topoisomerase IIα and β[119]. Multiple sequence alignments between species revealed a striking degree of similarity between the type II DNA topoisomerases of different species, including rat, mouse, hamster, drosophila, yeast, bacteria and viruses[120]. In addition, *Wasserman et al.* have demonstrated that yeast can be used in the study of anticancer drugs targeting human DNA topoisomerase IIα[121].

The two mammalian topoisomerase II isoforms, α and β possess a high degree of amino sequence identity (~70%) while being encoded by different genes. However, they are expressed differently during growth cycle. Whereas topoisomerase IIβ appears to be present in most cell types, topoisomerase IIα concentrates in rapidly proliferating cells, while being almost nonexistent in resting tissues. Prokaryotic type II topoisomerases are heterodimers (A_2B_2), whereas eukaryotic analogs are homodimeric (A_2) in nature. Over the course of evolution the *ParC* and *ParE* parts of bacterial enzyme fused into single protein, encoded by single gene in mammalians[122].

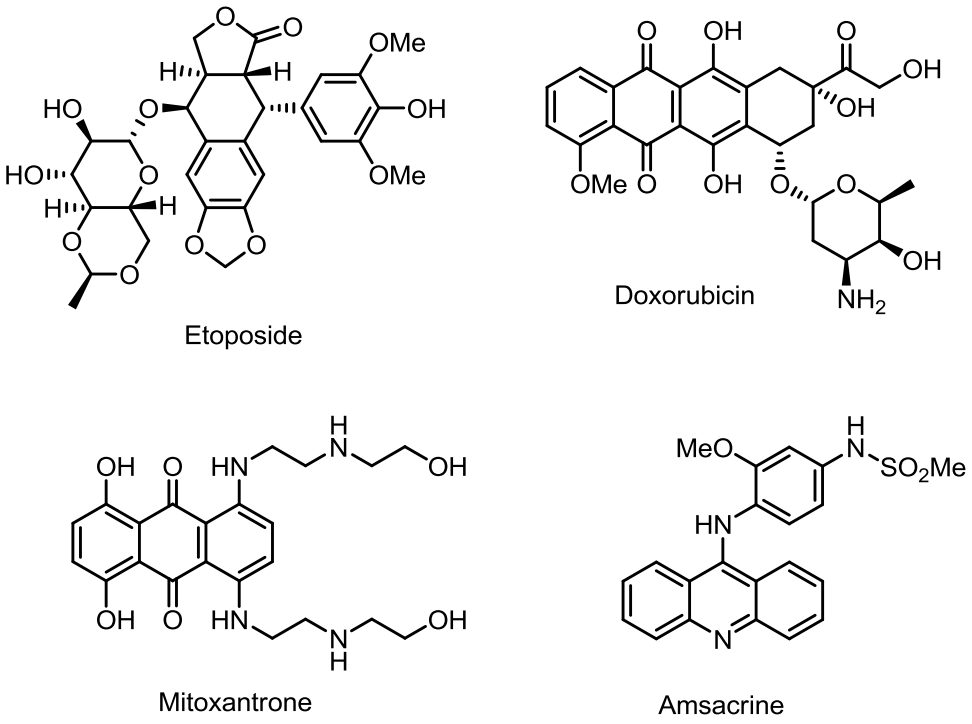


Figure 11 Representative examples of anticancer drugs acting through topoisomerase II inhibition

Mammalian topoisomerase II is a target for a number of anticancer drugs, such as etoposide, doxorubicin, mitoxantrone and amsacrine (**Figure 11**)[123]. These drugs can be divided into inhibitors and poisons. While topo II poisons stabilize specifically covalent DNA-topoisomerase complex (cleavage complex), topo II inhibitors act at any other step of catalytic cycle. Topo II inhibitors bind the enzyme, thus excluding it from catalytic cycle. This blocks DNA related processes, like replication, translation and transcription and causes some cytostatic effects[124]. Topo II poisons can act in two possible ways: they could block enzyme in the ternary complex with torn DNA and inhibit religation of DNA breaks or increase the forward rate of topoisomerase II-DNA cleavage complex formation, in each case producing double-stranded breaks in DNA. The presence of numerous double-stranded breaks starts up a repair mechanism, which tends to provoke single-nucleotide exchanges or single-base deletions. In such way topo II poisons turn topoisomerase into endogenous toxin which causes damage to genetic material. This triggers a sequence of biochemical events leading to a programmed cell death, apoptosis[125].

Quinolone derivatives with postulated action as topo II poisons are CP-115,953[116a, 126] , WIN-58,161[127] and SNS-595[128], while A-62,176 is likely to be a catalytic topo II inhibitor[129] (**Figure 12**).

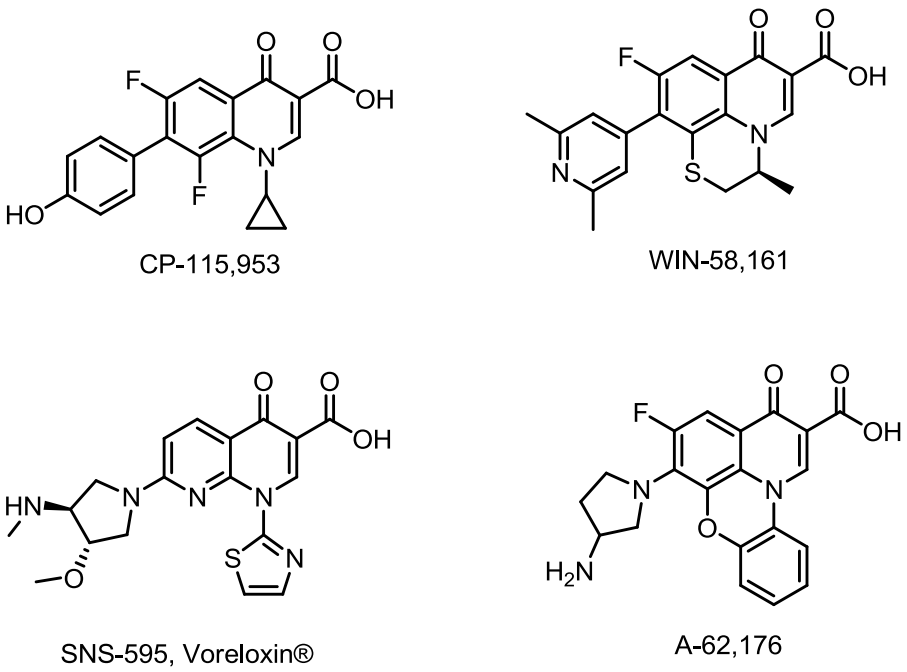


Figure 12 Representative examples of quinolone topo II inhibitors and poisons

3.2.2 Topoisomerase I

A number of currently used antitumor drugs such as camptothecin derivatives, topotecan and irinotecan target topoisomerase I[130]. This enzyme has functions similar to those of topoisomerase II; it relieves tension in duplex DNA by introducing a temporary, enzyme-bond, single-strand break in the DNA double-helix. The resulting nick permits rotation of the downstream helix about one or more bonds in the intact strand that is driven by the free energy associated with DNA supercoiling. Topo I targeted drugs act by trapping enzyme-DNA covalent complex and by reducing the rate of religation they prolong the lifetime of nicked intermediated. It is generally believed that lethal action of these drugs is mediated through stabilization of topoisomerase-DNA complex that could produce lethal double-stranded breaks when encountered by DNA replication enzymes[131]. *You et al.*[132] designed, synthesized and evaluated a series of 32 quinolone derivatives as potential topoisomerase I inhibitors. The 2 most potent compounds (**Figure 13**) presented a significant inhibitory effect on topo I, was proved to induce cell death via apoptosis and accelerated DNA strand breaks without significant alteration in cell cycle populations.

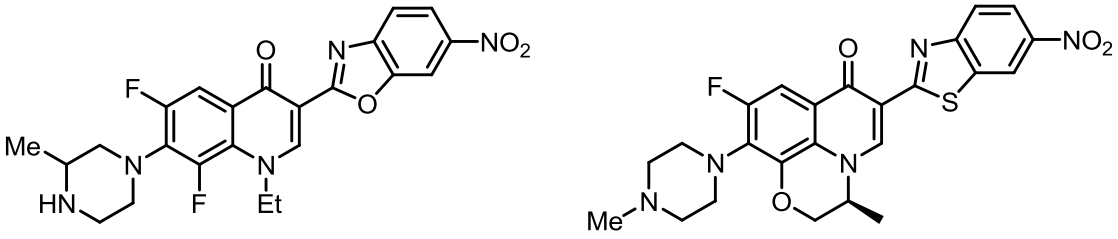


Figure 13 Quinolone-based topo I inhibitors synthesized by *You et al.*[132]

3.2.3 G-quadruplex structures

During the course of extensive search for potent anticancer drugs new targets recently started to emerge. G-quadruplex structures are known to inhibit telomerase activity by sequestration of the substrate required for enzyme activity[133]. Therefore small organic molecules with planar structure that stabilize or induce G-quadruplex structures have been suggested as potential anticancer therapeutic agents. Indeed, research in this direction led to discovery of the fluoroquinonaphtoxazine CX-3543 which is the first G-quadruplex interactive agent to enter human clinical trials. This molecule is currently under evaluation against carcinoid/neuroendocrine tumors in a phase II clinical trial[118]. Various FQs with attached naphtoxazine or anthroxazine rings and planar structure are able to interact with duplex DNA and to intercalate with the more expansive planarity of G-quadruplex structures[116b, 134] (**Figure 14**). CX-3543 binds to G-quadruplex DNA and was shown to selectively disrupt interaction of rDNA G-quadruplexes with the nucleolin protein thereby inhibiting RNA Polymerase I complex transcription and inducing apoptotic death in cancer cells[135].

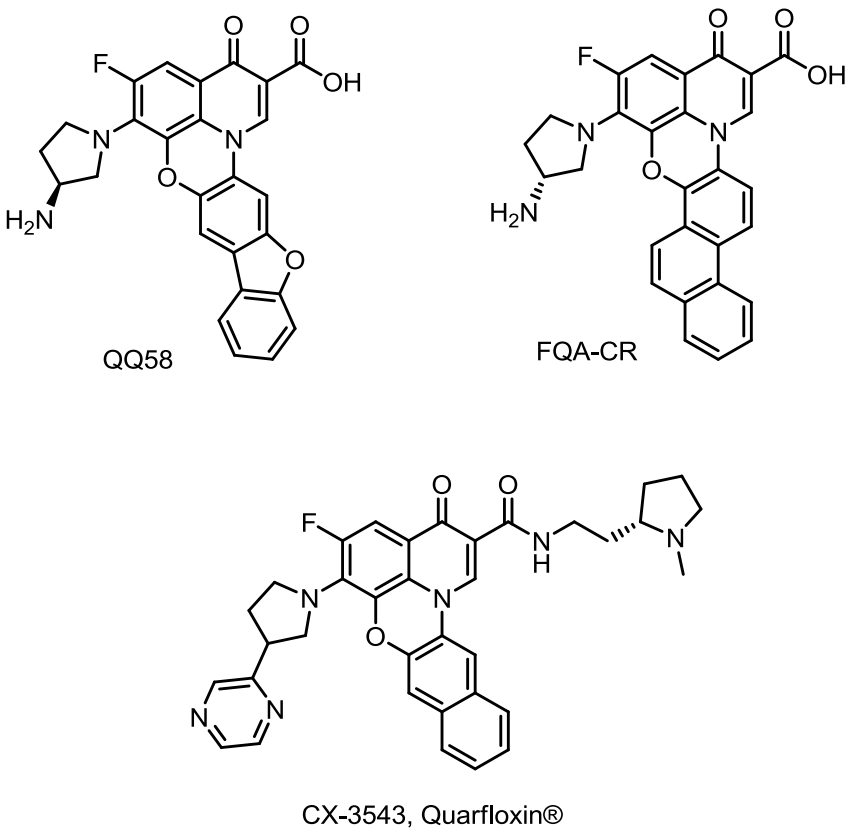


Figure 14 Representative quinolone-based G-quadruplex inhibitors

3.2.4 Microtubules

Microtubules are intracellular targets for anticancer chemotherapy. These fibrillar structures play important role in a variety of cellular processes, in particular the cell mitosis. Microtubules are formed as a result of polymerization of α- and β-tubulin with microtubule associated proteins. Wide range of drugs could interfere with normal formation of microtubule and mitotic spindle thereby provoking the cell cycle arrest in the metaphase[136]. Compounds that target microtubules are usually divided into stabilizers and destabilizers. Microtubule stabilizers are represented by drugs from taxane family, paclitaxel and docetaxel, along with the natural epothilones and their analogs (**Figure 15**).

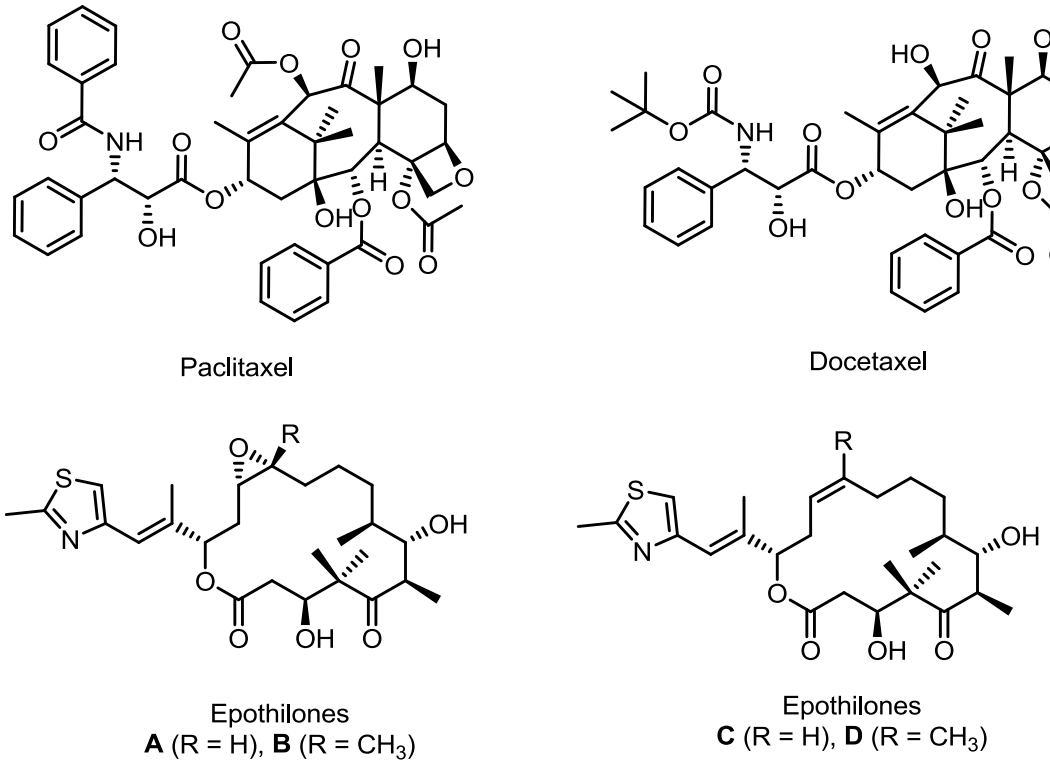


Figure 15 Microtubule stabilizing anticancer agents

Theses drugs bind to microtubule and prevent it from disassembly which causes cell death by apoptosis[137]. Microtubule destabilizers predominantly bind at central β-tubulin subunit and thus prevent it from polymerization[138]. Quinolone agents who showed significant antimitotic activity are generally represented by 2-aryl derivatives (NSC 664171[139], CHM-1[140], YJC-1[141], **Figure 16**). These microtubule destabilizers inhibit the tubulin polymerization, leading to mitotic arrest in G2/M phase and induce apoptosis by Cdk-1 involved signaling pathways[142]. In this series the most potent representatives demonstrated *in vitro* nanomolar IC_{50} and reached preclinical trials in humans.

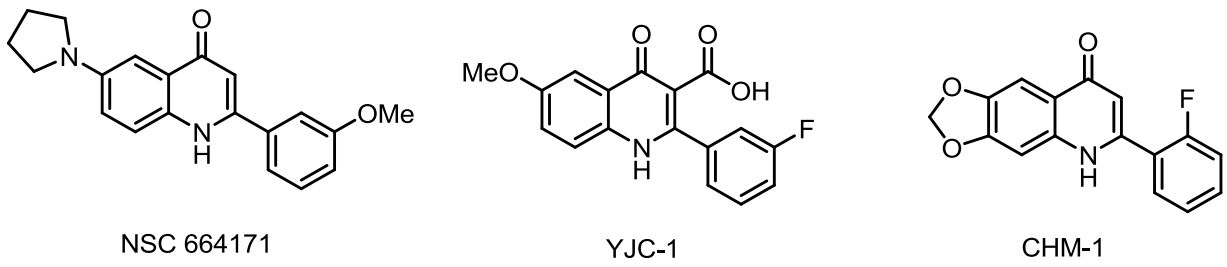


Figure 16 Representative examples of antimitotic quinolones

3.3 Structure-activity relationships

In contrast to the quinolone antibacterials with thousands of tested compounds and detailed SAR studies, quinolone-based anticancer agents only started to emerge with a few most potent representatives. There are numerous obstacles to define SAR for cytotoxic quinolones, like the variety of intracellular targets and a large diversity of tested cancer cell line types. Moreover, similar to the previously described antibacterial SARs, no single position has a controlling effect on the observed antitumor activity but, more likely, a combination of various substituents contributes to the cytotoxic potency. So, in order to maintain clarity and reliability, one should account for the type of cancer cell line used, biological target and the nature of other substituents presented in the molecule. In the following sections quinolone structures will be discussed separately for each target (if known) and a last paragraph will be devoted to "cytotoxic quinolones" with unidentified target.

3.3.1 Topoisomerase II poisons and inhibitors

In vitro binding studies have revealed a common domain of interaction between eukaryotic topoisomerase II with both antineoplastic inhibitors such as etoposide and FQs[143]. However, the FQ concentrations required *in vitro* to form a cleavage complex with native eukaryotic type II topoisomerase were 100- to 1000-fold higher than those required for gyrase[114-115, 144]. Initial studies were directed by attempts to shift inhibitory activity from bacterial to eukaryotic topoisomerase II. As a result of these reports several compounds with high potency were identified and SARs were established. A schematic historical development of anti-topoisomerase II quinolones is reported in **Figure 17**.

New structures with C-7 aryl substituents were first identified and the antibacterial lead compound Rosoxacin was transformed into cytotoxic CP-67,015 by addition of two fluorine atoms at C-6 and C-8 positions[115]. Further optimization led to CP-67,804 and CP-115,953 with 4-hydroxyphenyl substituent at C-7 position. These compounds were both cytotoxic *in vitro* and number of experimental evidences indicated that they target topoisomerase II[116a].

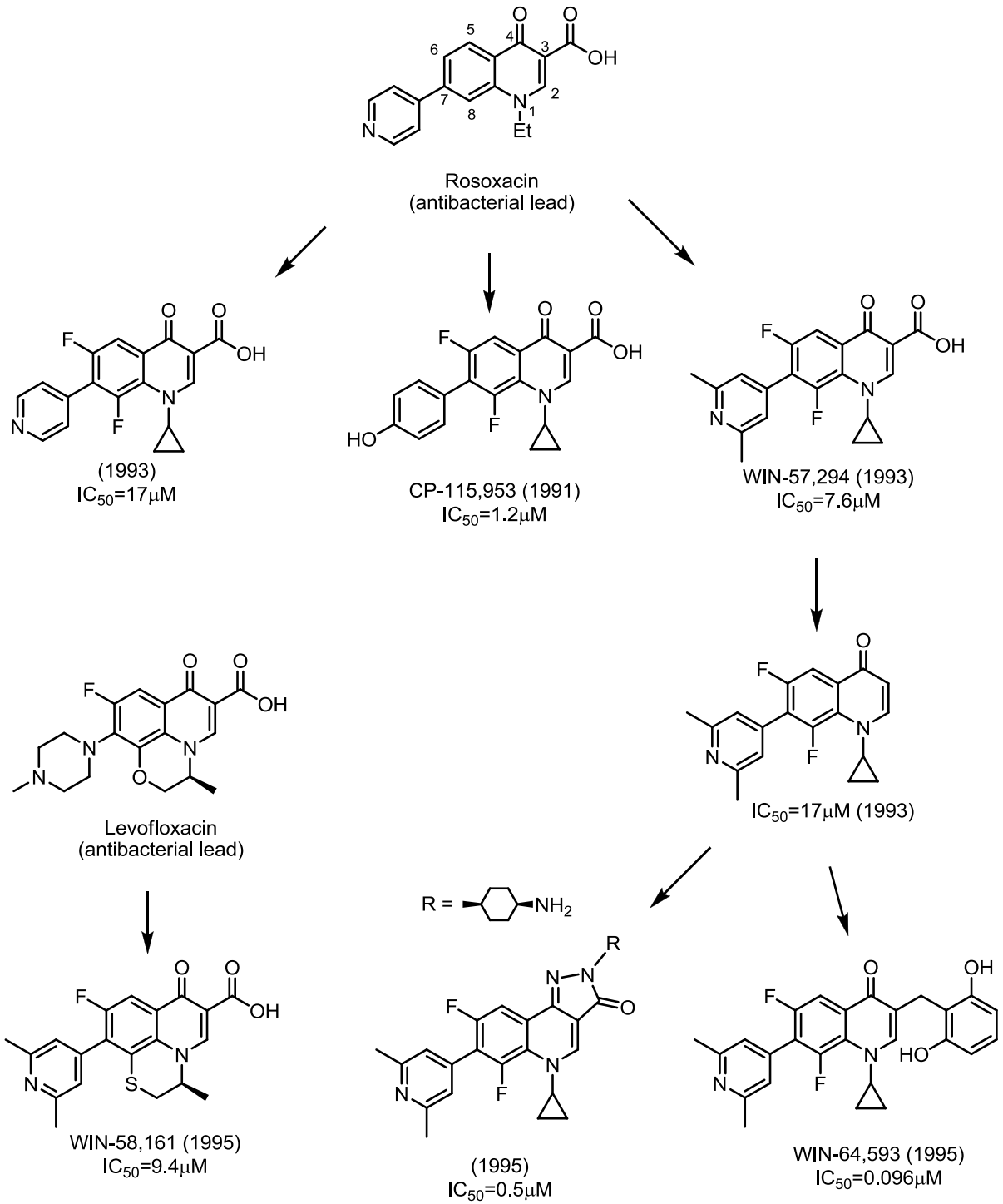


Figure 17 Schematic development of anti-topoisomerase II quinolones (IC_{50}, concentration that inhibits human HeLa topoisomerase II-DNA complex formation by half).

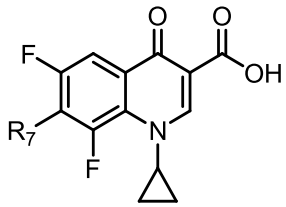
The discovery of C-7 aryl cytotoxic quinolones lead to SAR concerning the role of C-7 substituent[144]. Examples shown in **Table 28** illustrate some general trends:

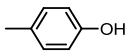
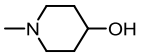
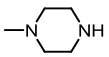
1) C-7 position is of high importance, in comparison to C-8 and N-1 positions

2) 4-Hydroxyphenyl substituent at C-7 position increase anti-topoisomerase II activity but also increase the cytotoxicity of compounds

It is worth to note that a clear correlation exists between *in vitro* topoisomerase II inhibitory activity, DNA cleavage and cytotoxicity. This supposes that the common biological target of these derivatives is topoisomerase II, in spite of structural differences at C-7 position.

Table 28 Anti-topoisomerase II activity and cytotoxicity of various C-7 modified CP-115,953 analogs

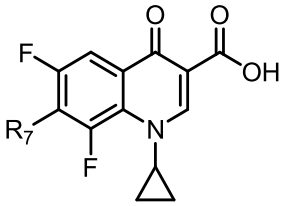


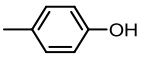
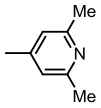
Compound	*R_7*	*DNA cleavage, EC_2 (μM)[a]*	*DNA relaxation, IC_{50} (μM)[b]*	*Cytotoxicity, CC_{50}(μM)[c]*
CP-115,953		0.3	3.2	9
CP-162,600		105	198	>500
CP-171,472		53	140	104
Difluorociprofloxacin		63	132	235

Data from ref [144] [a]EC_2, Effective concentration of agent required to enhance calf thymus topoisomerase II-mediated DNA cleavage twofold [b]The agent concentration required to inhibit DNA relaxation by calf thymus topoisomerase II by 50% [c]Concentration of compound required to kill 50% of Chinese hamster ovary (CHO) cells.

At the same time *Wentland et al.* studied a series of pyridine based C-7 substituents and their influence on topoisomerase II inhibition[145]. The authors demonstrated that 4-pyridinyl moiety at C-7 position could also give rise to cytotoxic compounds; however, their levels of potency were lower than that of 4-hydroxyphenyl analog (CP-115,953) and tuning with additional methyl groups is needed to increase the topoisomerase II inhibitory activity (**Table 29**).

Table 29 Pyridinyl vs phenyl substituent at C-7 position with regard to anti-topoisomerase II activity

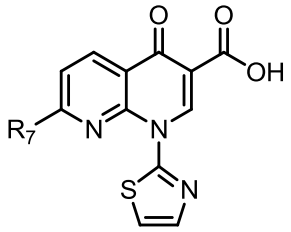


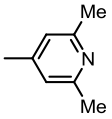
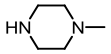
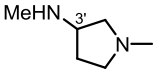
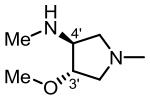
R_7	IC_{50} (μM)[a]
 (CP-115,953)	1.2
	17
 (WIN-57,294)	7.6

Data from ref [145] [a]Concentration that inhibits human HeLa topoisomerase II-DNA complex formation by half

During the initial studies SNS-595 didn't induce topoisomerase II-mediated DNA cleavage, thereby it wasn't supposed to act as a topoisomerase poison[146]. However, detailed studies established that voreloxin intercalates DNA and poisons topoisomerase II, inducing site-selective double-strand DNA breaks and G2 arrest[128]. During the development of SNS-595 large number of derivatives bearing different groups at C-7 position were prepared and evaluated. Some SARs were established and, particularly, aminopyrrolidine moieties at C-7 provided promising cytotoxic activity against murine P388 leukemia cells. Analysis of different substitution patterns revealed that additional substitution at 4'-position of pyrrolidine cycle further increases the cytotoxicity. Among all, 3'-methoxy-4'-methylaminopyrrolidinyl analog showed the highest cytotoxic activity in both *in vitro* and *in vivo* assays[146b].

Table 30 *In vitro* antiproliferative activity of SNS-595 and its C-7 analogs against murine P388 leukemia cells



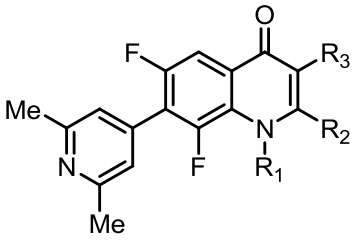
R_7	IC_{50} (μM)[a]
	18
	19
	0.16
	0.028
	0.019
SNS-595 (®Voreloxin)	

Data from ref [146b] [a]Concentration of agent that reduces murine P388 leukemia cell viability by 50%

In the previously discussed work, *Wentland et al.*[145] also studied the influence of N-1 and C-3 positions on overall potency. Although all compounds were less active than CP-115,953 the authors demonstrated that (**Table 31**):

1) Ethyl and unsubstituted N-1 position are not tolerated, with cyclopropyl being the optimal for topoisomerase II inhibition;
2) The presence of 3-CO_2H group is not necessary for high levels of anti-topoisomerase II activity and 3-H analog was also significantly active;
3) In the absence of C-3 carboxylic group, C-2 position could be substituted with small groups (CH_3) without loss of activity, while larger phenyl derivative is inactive

Table 31 Influence of R-1, R-2, R-3 substituents on human topoisomerase II inhibition



R_1	R_2	R_3	$IC_{50,}(\mu M)^a$
c-Pr (WIN-57,294)	H	CO_2H	7.6
Et	H	CO_2H	110
H	H	CO_2H	>300
c-Pr	H	H	17
c-Pr	CH_3	H	16
c-Pr	Ph	H	>240

Data from ref [145] [a]Concentration that inhibits human HeLa topoisomerase II-DNA complex formation by half

This unexpected result contrasts the SAR determined in quinolone antibacterials, where the 3-CO_2H (or isosteric replacement) is required for prokaryotic topoisomerase inhibition.

Interestingly, these results induced further structural exploration at C-3 position, leading to the discovery of WIN-64,593. This 2,6-dihydroxybenzyl derivative is a topoisomerase II inhibitor[147] (**Figure 17**, **Figure 18**) with IC_{50} of 0.096 μM (80-fold more active than parent WIN-57,294) and *in vivo* antitumor properties in mice.

In addition, a series of compounds with annulated cycles at C-3 position showed enhanced cytotoxicity against mammalian cells. Studies have shown that the requirement for inhibition of eucaryotic enzyme seems to be the coplanarity of the C-3 substituent with the quinolone. Isothiazoloquinolones A-65,281 and A-65,282, (**Figure 18**) inhibited DNA unknotting with 50% inhibitory concentrations of 21 μM and were found to induce significant DNA breakage mediated by calf thymus topoisomerase II[78]. Another interesting example of successful C-3 modification is represented by derivatives bearing a pyrazolo-ring fusion at the 3- and 4-positions of the quinoline moiety (**Figure 18**). The most potent pyrazoloquinoline displayed human HeLa topoisomerase II IC_{50} = 0.5 μM, **Figure 18**), and demonstrated antitumor activity both *in vitro* (P388 mouse leukemia cells) and *in vivo* (mice)[148].

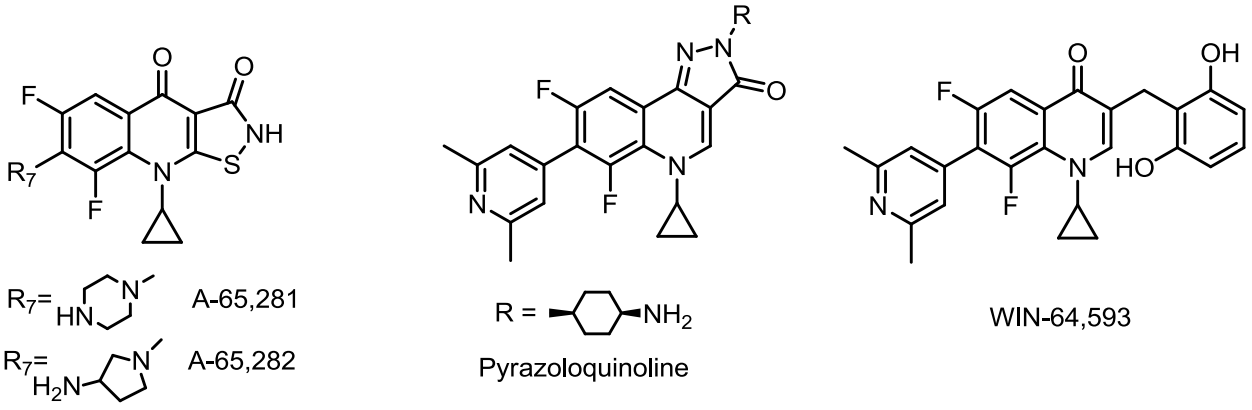
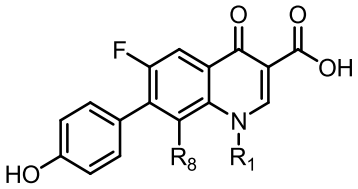


Figure 18 Representative examples of anti-topoisomerase II quinolones with modified C-3 position

Robinson et al.[149] conducted a detailed study concerning the role of C-8 fluorine and N-1 cyclopropyl substituents. The authors showed that, in comparison with CP-115,953, defluorinated analog CP-115,955 (**Table 32**) had lower levels of DNA cleavage and topoisomerase II inhibition which is thought to be related with lower *in vitro* cytotoxicity towards mammalian cells. Moreover, CP-115,953 with N-1 cyclopropyl substituent turned out to be more potent than N-1 ethyl analog CP-67,804 (**Table 32**). Some levels of cross resistance to quinolones in etoposide-resistant cell line (etoposide also targets topoisomerase II) suggested that topoisomerase II is a target for these quinolones).

Table 32 Influence of C-8 fluorine and N-1 substituents on topoisomerase II activity and cytotoxicity



Compound	*R_1*	*R_8*	*Topoisomerase II activity*[a]		*Cytotoxicity CC_{50} (μM)*[d]	
			DNA cleavage[b]	*IC_{50} (μM)*[c]	*Wild type*	*Vpm^R-5*[e]
CP-115,953	c-Pr	F	1,00	60	9	12
CP-115,955	c-Pr	H	0.38	130	20	40
CP-67,804	Et	F	0.39	325	70	265

Data from ref [116a, 149] [a]*Drosophila melanogaster* topoisomerase II [b]Relative scale of potency with 1,00 value set for CP-115,953 [c]Concentration that inhibits topoisomerase II DNA relaxation ability by half [d]Concentration that reduces the proliferation of Chinese hamster ovary (CHO) cells by 50% [e]Selected cell line with resistance to etoposide

At the same time *Suto et al.*[150] investigated a large number of FQs for their cytotoxicity towards mammalian cells, which is thought to be related with topoisomerase II inhibition. In this report, halogens at C-8 and cyclopropyl at N-1 gave the most cytotoxic derivatives, which is consistent with previous results of *Robinson et al.*[149] (**Table 32**)

Numerous SARs were established during the development of SNS-595[146] from antibacterial lead compound AT-3639[151] (**Figure 19**).

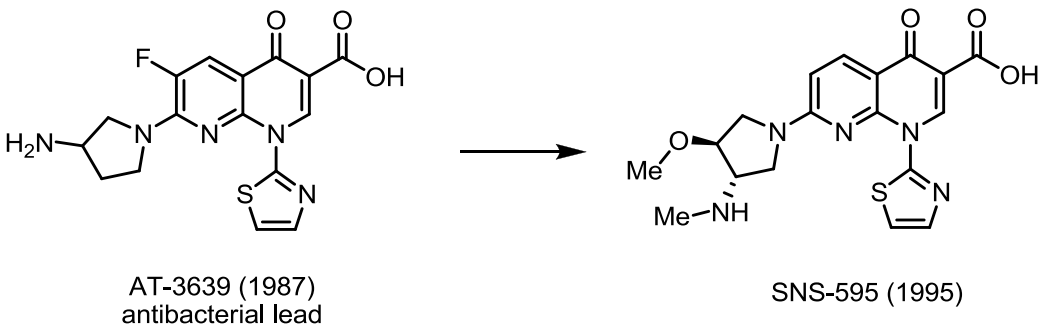
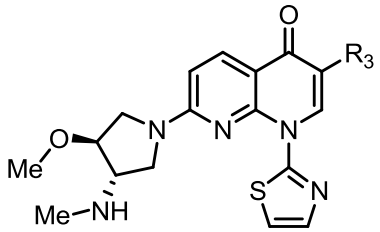


Figure 19 Development of SNS-595, promising antitumor quinolone

Interesting results were obtained by *Tsuzuki et al.*[151] when different C-3 substituents where introduced into SNS-595 molecule. The authors provided another prove for tolerating modifications at C-3 position with regard to cytotoxic activity. The analog bearing formyl group was almost equipotent to parent SNS-595 compound both *in vitro* and *in vivo* (mice implanted with P388 leukemia cells) (**Table 33**). However, low solubility of the formyl analog hampers its practical usage as a therapeutic agent. Notwithstanding these studies gave basis to the further exploration of more usefull antitumor drug candidates.

Table 33 *In vitro* cytotoxicity and *in vivo* antitumor activity of C-3 modified SNS-595 analogs

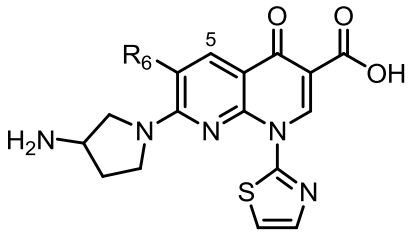


R_3	*In vitro*	*In vivo*	
	IC_{50}[a] (μM)	*Dose, mg/kg*	*T/C*[b], *%*
CO_2H (SNS-595)	0.01	3.13	200
CHO	0.03	3.13	188
CH_2OH	0.06	–	–

Data from ref [151] [a]Concentration of agent that reduces murine P388 leukemia cell viability by 50% [b](Median survival time of treated mice)/(median survival time of controls)×100.

While exploring C-6 substituent *Tomita et al*[146a] reported that non-fluorinated analog was twofold more cytotoxic against murine P388 leukemia cells than fluorinated AT-3639 (**Table 34**). In comparison with the 6-F counterpart, 6-NO_2, 6-NH_2 and 6-OH derivatives were significantly less active (IC_{50} values were 5.1; 2.4; 10 μM respectively). In addition, the authors demonstrated that further modifications at C-5 position of the 6-unsubstituted 1,8 naphthyridone lead to less cytotoxic activity, with exception of 5-NH_2 derivative which was almost equipotent to the unsubstituted compound with IC_{50} = 0.019 μM.

Table 34 Cytotoxic activity of C-6 fluorinated vs unsubstituted 1,8 naphthyridones against murine P388 leukemia cells



R_6	IC_{50}^{a} (μM)
H	0.028
F (AT-3639)	0.056

Data from ref [146a] [a]Concentration of agent that reduces murine P388 leukemia cell viability by 50%

The shift from bacterial to mammalian topoisomerase inhibition activity was also noted for LEV analogs. *Wentland et al.*[127, 152]showed that the benzothiazene-6-carboxylic acid WIN-58,161 (**Figure 20**) displayed significant inhibitory potency for mammalian HeLa topoisomerase II. Interestingly enough, the anti-topo II activity was strongly dependent on the stereochemistry of C-3 atom, with the preference for the *S*-enantiomer (**Figure 20**). The same preference was previously observed for *E. coli* gyrase inhibition by LEV (*S* enantiomer of OFL)[50]. The authors also showed that replacing the sulfur atom of WIN-58,161 with oxygen decreased the potency 3-fold (**Figure 20**).

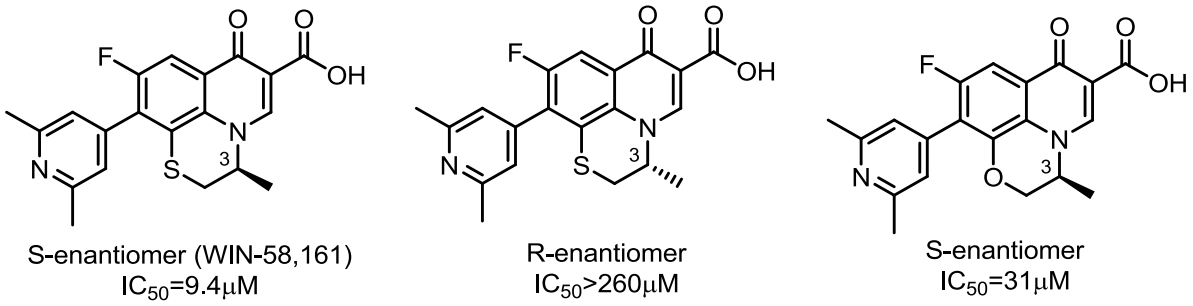
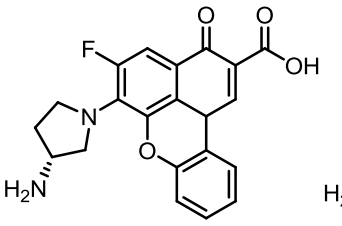


Figure 20 Influence of stereochemistry on human HeLa topoisomerase II inhibition[152]

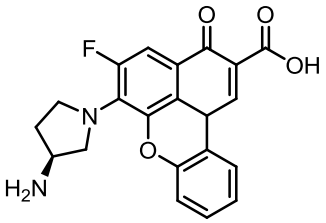
Another path of LEV modification is represented by quinobenzoxazines, a family of cytotoxic quinolones (**Table 35**). The attachment of benzene ring to oxazine moiety provokes shift from bacterial to mammalian topoisomerase inhibition. Moreover, the mode of action is

different. Quinobenzoxazines don't mediate topoisomerase II dependent DNA double-strand breaks. Instead, the *in vitro* cytotoxicity of compounds correlates well with both topoisomerase II inhibition and DNA binding[153] (**Table 35**). Thus, the authors supposed that quinobenzoxazines are catalytic topoisomerase II inhibitors[129] which act before DNA cleavage stage in contrast to previously mentioned topo II poisons[154].

Table 35 Quinobenzoxazines: relationship between *in vitro* cytotoxicity, DNA binding and topoisomerase II inhibition



(R)-A-62,176



(S)-A-62,176

Compound	*Cytotoxicity* CC_{50}, μM[a]	*DNA binding*[b]	*Topoisomerase II inhibition* IC_{50}, μM[c]
(R)-A-62,176	0.89	++	3.1
(S)-A-62,176	0.53	+++	0.9

Data from ref [153b] [a]Concentration of compound that reduces A-549 cell viability by 50% [b] (+++) – compound fully protects DNA sequence from DNase I at 1 μM; (++) – compound fully protects DNA sequence from DNase I at 10 μM [c]Concentration of compound that inhibits kinetoplast DNA decatenation by human topoisomerase II by 50%

3.3.2 G-quadruplex inhibitors

G-quadruplex inhibitors, in order to effectively interact with nucleotide bases, should possess extensive degree of planarity. Historically, these molecules evolved from antibacterial lead LEV by step-wise annelation of flat aromatic cycles (**Figure 21**).

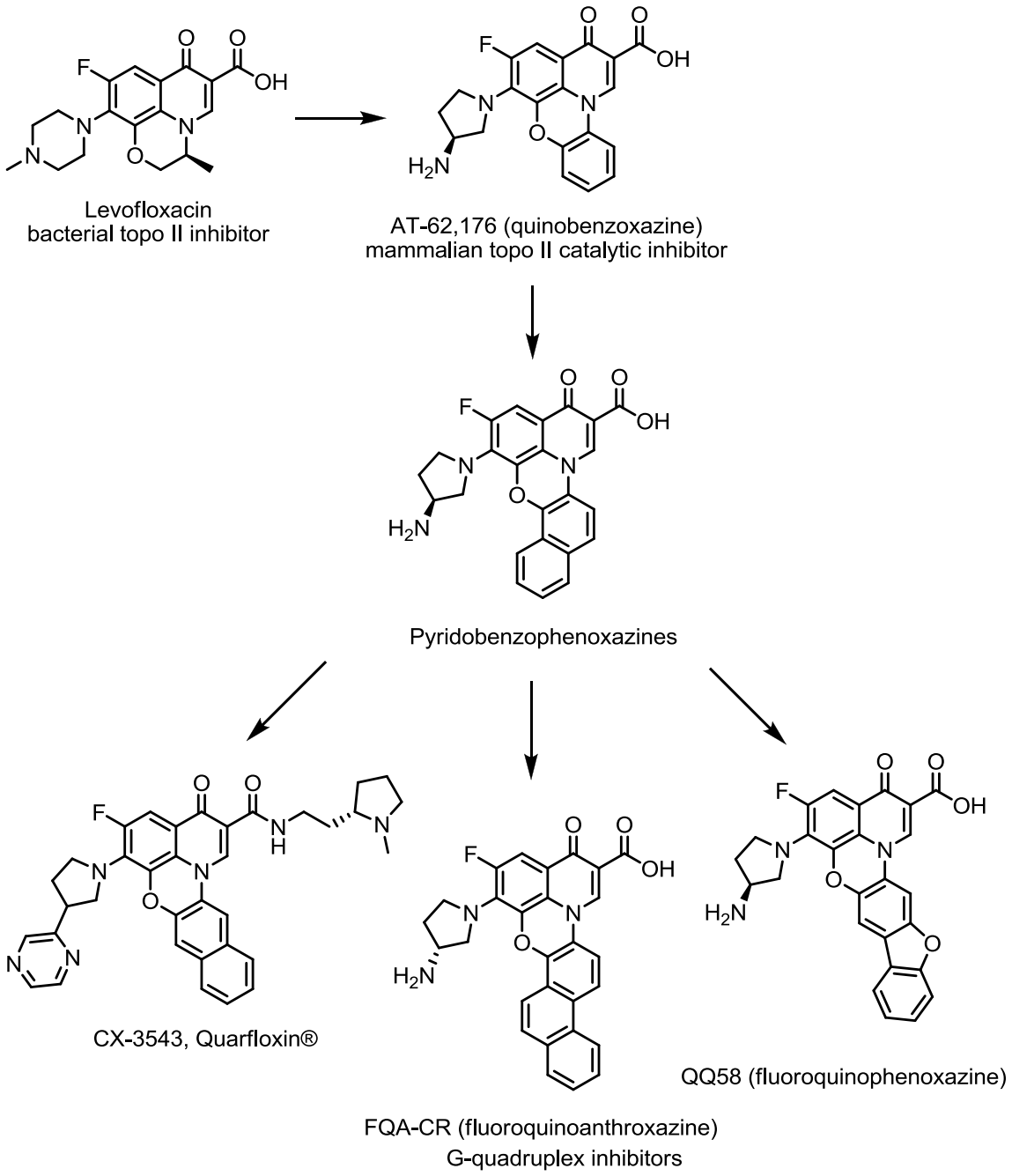
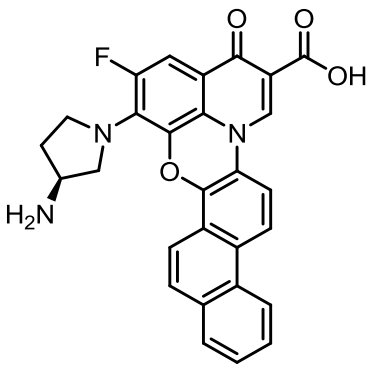


Figure 21 Development of G-quadruplex inhibitors

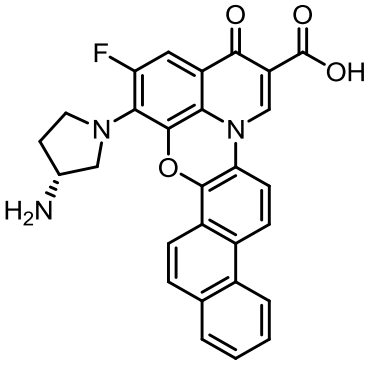
The quinobenzoxazine *(S)*-A-62,176 termed AT-62,176, a mammalian cytotoxic agent, was shown to be both a topoisomerase II catalytic inhibitor[129] and poison[154]. The pyridobenzophenoxazine analogs, with an expanded ring system, also showed strong cytotoxic effects on several tumor cell lines and potent inhibitory effects against human topoisomerase II[153a]. CX-3543[135] and the fluoroquinophenoxazine QQ58[116b] both demonstrated enhanced stacking interactions with G-quadruplex, but had lost the topoisomerase II poisoning activity. On the other hand, the fluoroquinoanthroxazines, containing a naphthyl ring in the *cis* orientation,

were able to recognize and bind quite specifically to G-quadruplex structures and topoisomerase II-DNA complexes. Within this group, FQA-CR showed a higher G-quadruplex interaction, while FQA-CS displayed more potent topoisomerase II poisoning activity (**Table 36**)[134].

Table 36 Biological activities of fluoroquinoanthroxazines



FQA-CS



FQA-CR

	FQA-CS	*FQA-CR*
G-quadruplex interaction: polymerase stop assay (IC_{50}, μM)	0.67	0.06
Topoisomerase II inhibition: cytotoxic ratio topoisomerase II-R/-S cells[a]	4.02	1.55
Cytotoxicity: MCF7 breast cells (IC_{50}, μM)	1.1	0.46
Major mechanism of action	topoisomerase II poison	G-quadruplex interaction

Data from ref [134] [a]The ratio of cytotoxicity of FQA-CS and FQA-CR against 8226/DOX1V (topoisomerase II-resistant) and 8226/S (topoisomerase II-sensitive) cells.

3.3.3 Antimitotic agents

The key structural features which shifted quinolone's biological action from topoisomerase II poisoning to inhibition of tubulin polymerization are C-2-aryl substituent and unsubstituted N-1 atom[155]. A large tolerance for aromatic groups at C-2 position was demonstrated and carboxylic group at C-3 position serves to improve activity and pharmacokinetics[141]. The C-6 substituent plays very important role for antimitotic activity and generally, the groups attached at C-6 should be lipophilic and electrondonating, such as methyl, hydroxymethyl, fluor and some cyclic amines. Other positions like C-5, C-7 and C-8 are

preferred to be unsubstituted (**Figure 22**). The most potent representatives of antimitotic quinolones were previously shown on the **Figure 16** (page 66)

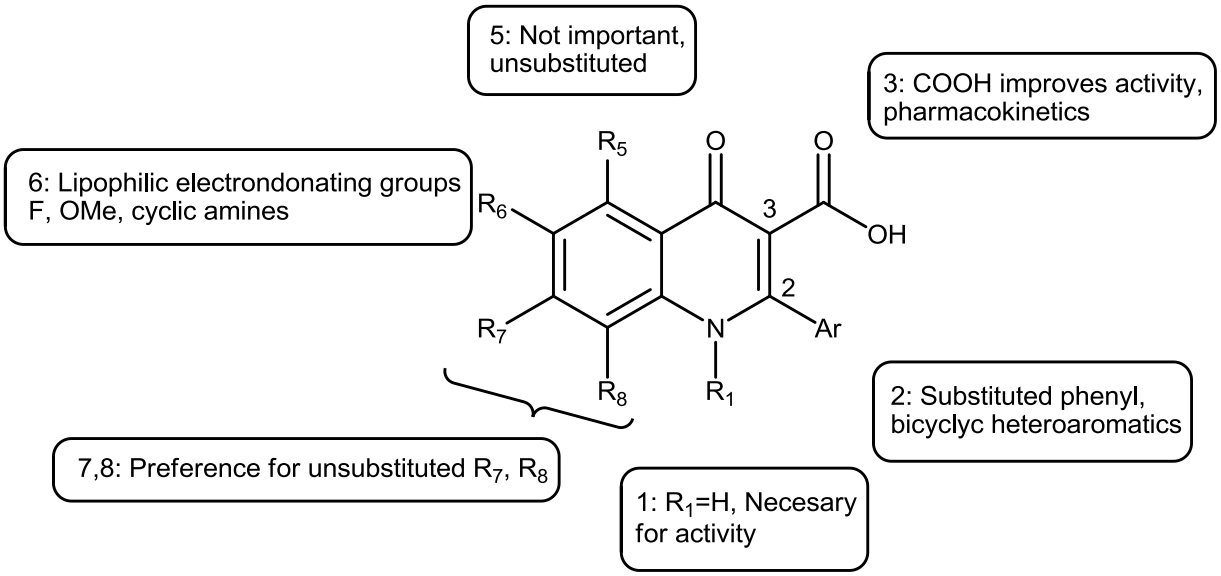


Figure 22 Summary of SAR for antimitotic quinolones

3.3.4 Cytotoxic quinolones

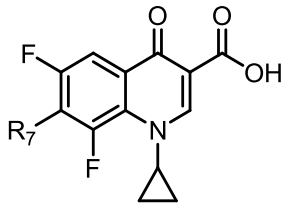
In this section we decided to present cytotoxic agents with "unidentified mechanism of action". This paragraph discusses the influence of modifications at C-3 and C-7 positions on cytotoxicity.

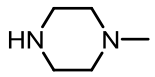
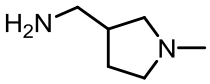
Detailed investigation of C-7 piperazinyl and pyrrolidinyl substituents was held by *Yamashita et al.*[156] and *Suto et al.*[150]. A large number of derivatives were evaluated for antitumor activity both *in vitro* and *in vivo* and the authors showed that:

1) pyrrolidinyl based derivatives are generally more cytotoxic *in vitro* (an example is presented **Table 37**)

2) piperazinyl and pyrrolidinyl based derivatives have comparable activities *in vivo*

Table 37 Evaluation of C-7-piperazinyl vs pyrrolidinyl cytotoxic quinolones



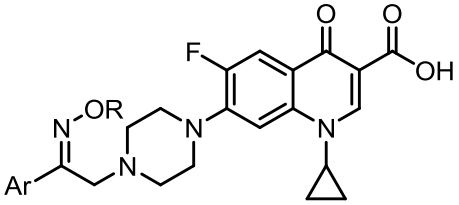
R7	*In vitro cytotoxicity IC50 (μM)*[a]	*In vivo antitumor activity ILS %*[b]
	134	30
	30	34

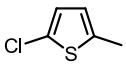
Data from ref [150, 156] [a]Concentration of compound that reduces Chinese hamster V79 cells viability by 50% [b]Increased life span (ILS) in murine P388 leukemia model

Recently novel substitutions were introduced at C-7 position, which can provide good models for further design of potent antitumor quinolones. Substitution of distal nitrogen atom at piperazine moiety provides significant improvement in cytotoxicity. Initial findings of *Fang et al.* [157] demonstrated that 7-[4-(2-hydroxyiminoethyl)piperazin-1-yl] quinolones displayed significant activities against renal cancer cell lines, among them, 7-4-[2-(4-chlorophenyl)-2-hydroxyiminoethyl]-1-piperazinyl-derivative was the most potent. *Foroumadi et al.* identified new aromatic moieties (furan-2-yl and thiophen-2-yl) that provide highly cytotoxic compounds[158]. In addition, the cytotoxicity was further enhanced by converting the oxime group to the respective O-benzyl or O-4-chlorobenzyl oxime[159] (**Table 38**).

To analyse the data authors have utilized the similar approach as in the screening program of National Cancer Institute with the panel of 60 human tumor cell lines used to screen for the antiproliferative activity. During these tests a new method of getting an idea on the substance's mechanism of action was discovered. In fact, the pattern of growth inhibition in these cell lines was similar for compounds of similar mechanism of action[160]. In the current article the *Foroumadi et al.* have compared log IC_{50} values of synthesized compounds with those of etoposide against less numerous panel of cancer cell lines. Finally they suggested that these quinolone derivatives share a similar mechanism of action with etoposide, already mentioned topoisomerase II inhibitor (**Table 38**).

Table 38 Cytotoxic oxyiminoethyl CIP derivatives

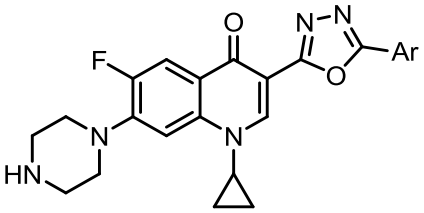


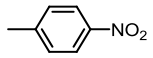
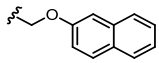
Ar	*R*	*Cytotoxicity IC_{50}, μM*[a]			
		MCF-7[b]	*A-549*[c]	*SKMEL-3*[d]	*KB*[e]
	H	117	108	125	108
	Me	116	111	127	102
	4-Cl-Bn	3.6	2.1	3.1	2.3
	Bn	1.8	1.6	3.3	1.9
	Me	2.9	–	7.7	17
CIP		199	–	181	160
Etoposide		11.6	1.5	0.34	4.8

Data from ref [158-159] [a]Concentration of compound that reduces cell viability by 50% [b]Breast carcinoma cell line [c]Lung cancer cell line [d]Melanoma cell line [e]Cervical carcinoma cell line

Concerning C-3 position 3-oxadiazole-aryl derivatives showed moderate cytotoxic activity against human tumor lung cancer cells (A-549)[161]. It is worth to note, that CIP derivatives were more potent than corresponding NOR analogs. The most active compounds bear *para*-nitrophenyl and β-naphtyloxymethyl substituents attached to the oxadiazole moiety (**Table 39**).

Table 39 Cytotoxic 3-oxadiazoyl quinolone derivatives

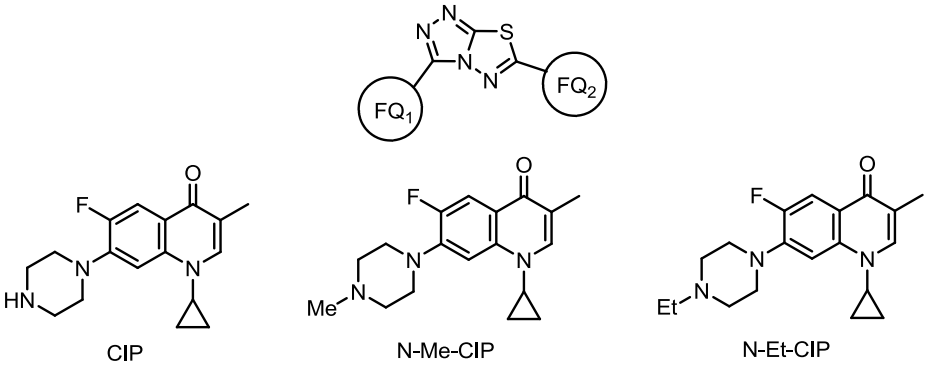


Ar	IC_{50} (μM)[a]
	1.7
	3.1

Data from ref [161] [a]Concentration of compound that reduces human lung tumor cell (A 549) viability by 50%

Analogously to antibacterials, cytotoxic quinolones were obtained by combining two pharmacophores through appropriate linker. By using fused heterocyclic triazolo[2,1-b][1,3,4]thiadiazole linker a series of "dimeric" FQ derivatives were prepared and showed *in vitro* cytotoxicity against CHO Chinese hamster ovary and L-1210 murine leukemia cell lines. The most potent compounds are CIP "heterodimers" presented in **Table 40**[162].

Table 40 C-3 linked cytotoxic FQ "dimers"



FQ_1	*FQ_2*	*IC_{50} (μM)[a]*
CIP	CIP	0.2
CIP	N-Me-CIP	1.2
CIP	N-Et-CIP	2.5

Data from ref [162] [a]Concentration of compound that reduces L210 (murine leukemia) cell viability by 50%

In summary, initial cytotoxic FQs developed from antibiotic agents exhibitied target shift from bacterial to mammalian topoisomerase inhibition. Studies revealed new potent stuctures which contrasted with previously established antibacterial SARs. The most active compounds possessed C-7 aryl substituents and, what is more interesting, some analogs exhibited structural modifications at C-3 position which was not tolerated in regard to antibacterial activity. Further research prompted the emergence of new structures for different targets, like G-quadruplex and tubulin polymerization inhibitors, therefore broadening the FQs spectrum of biological activity.

4. Conclusion

In this chapter we have described the different aspects concerning FQs, their emergence, development, mechanism of action and discussed their SARs for different biological targets. FQs represent an important group of molecules with crucial applications in medicine. However, old FQs displayed several drawbacks, such as emerging resistance, so the development of new agents is needed. For all these reasons, the synthesis of new FQ derivatives will be described in the next chapter along with the detailed rationalization for chosen compounds.

CHAPITRE II SYNTHÈSE DES DÉRIVÉS DE LA CIPROFLOXACINE ET DE LA LÉVOFLOXACINE

1. Introduction

Dans ce chapitre, nous décrivons le développement et la synthèse de nouveaux dérivés de la LEV et de la CIP.

Tout d'abord, le choix des composés synthétisés sera examiné. Nous discuterons les modifications structurales envisagées et la rationalisation pour l'introduction de différents substituants au niveau de l'acide carboxylique en position C-6 de la LEV et de la fonction amine N-4-pipérazine en position C-7 de la CIP.

Dans la deuxième partie, nous décrirons la synthèse des dérivés sélectionnés. Nous discuterons des travaux antérieurs effectués au laboratoire et nous détaillerons les différentes voies de synthèse choisies après optimisation.

La troisième partie de ce chapitre sera consacrée à l'étude de la stabilité des composés synthétisés dans un tampon phosphate aqueux, pH 7.4 à 37 °C, effectuée par HPLC.

2. Dérivés “monomères” de la ciprofloxacine

2.1 Introduction

Dans le premier chapitre, nous avons résumé quelques-uns des résultats de la littérature qui indiquent que la modification de la position C-7 des FQs ouvre des perspectives intéressantes pour le développement de nouveaux composés possédant des activités antibactériennes, antimycobactériennes ou antitumorales. En effet, l'inhibition de l'ADN topoisomérase par les quinolones est fortement influencée par la nature du substituant en position C-7. De plus, des modifications structurales en cette position auraient des effets sur la sélectivité enzymatique procaryotes versus eucaryotes. Certains auteurs ont montré que l'introduction de substituants volumineux au niveau du motif pipérazine des quinolones entraîne un déplacement de l'activité biologique antibactérienne de ces composés vers des agents cytotoxiques contre les cellules cancéreuses humaines[157-158]. Toutes ces caractéristiques ont suscité l'intérêt de notre laboratoire. Trois séries homologues de dérivés lipophiles de la CIP ont été conçues et synthétisées lors de travaux antérieurs dans notre groupe. Ces dérivés constituent une famille de dérivés 7-((4-substitué)pipérazin-1-yl)-CIP considérés comme des dérivés “monomères”. La CIP a été choisie pour sa disponibilité commerciale et la possibilité de fonctionnalisation de l’amine secondaire *N*-4-pipérazine. En outre, l’introduction de groupements de longueurs de chaîne variables pourrait permettre de moduler les propriétés physico-chimiques des composés et ainsi modifier le profil de l'activité biologique. Les trois séries de composés synthétisés diffèrent les unes des autres par la nature de la fonction reliant la chaîne alkyle à l'atome d'azote *N*-4 du groupement pipérazine (**Figure 23**).

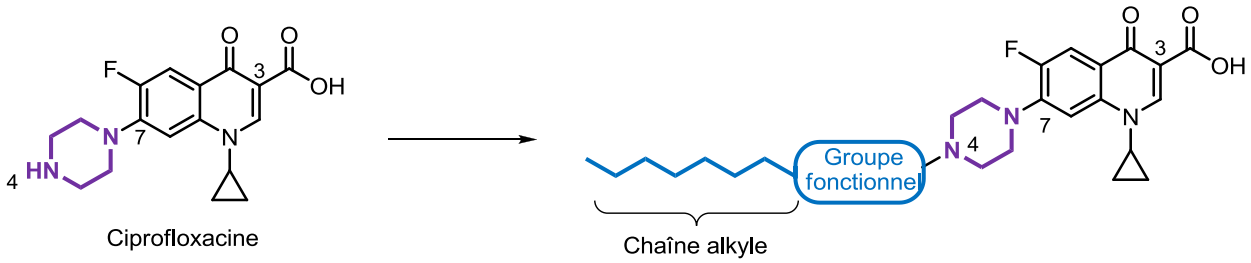


Figure 23 Représentation schématique des dérivés “monomères” 7-((4-substitué)pipérazin-1-yl)-CIP

Il est important de mentionner que la fonction acide carboxylique en C-3 de la CIP a une valeur de pKa élevé (6.08)[61] comparée aux autres aryl-acides. Cette fonction est en outre désactivée en raison de la formation d’une liaison hydrogène intramoléculaire avec la fonction

carbonyle adjacente en position 4 (**Figure 24**). Ainsi, aucune protection de l'acide carboxylique n'est nécessaire lors des synthèses pour la fonctionnalisation de la pipérazine de la CIP décrites dans la section suivante.

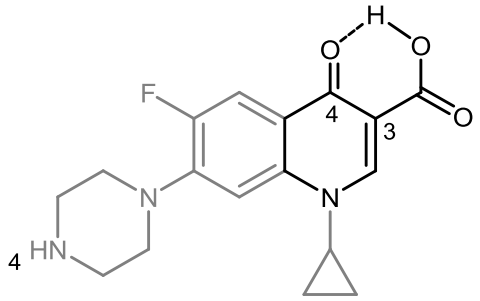


Figure 24 Liaison hydrogène intramoléculaire dans la molécule de CIP

2.2 Synthèse des dérivés 7-(4-(alcanoyle)pipérazin-1-yl) de la CIP

La première série de dérivés 7-(4-(alcanoyle)pipérazin-1-yl) de la CIP (dite "mono-amides") a été aisément préparée en une seule étape d'acylation de la CIP par les chlorures d'alcanoyle disponibles commercialement pour conduire aux composés correspondants **1a-l** avec de bons rendements variant de 45% à 90% (**Schéma 1**, **Tableau 41**). Certains dérivés de cette même série ont été difficiles à purifier, ce qui explique les différences entre les rendements.

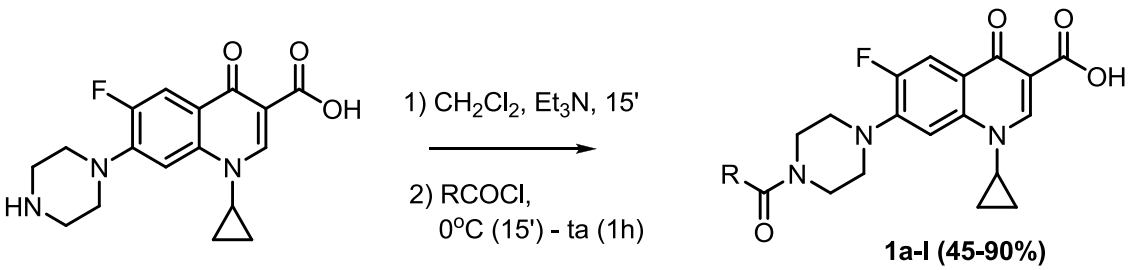


Schéma 1 Préparation des dérivés 7-(4-(alcanoyle)pipérazin-1-yl) de la CIP **1a-l**

Tableau 41 Rendements de synthèse des dérivés 7-(4-(alcanoyle)pipérazin-1-yl) de la CIP **1a-l** "mono-amides"[a]

Composé	*R*	*Rdt, %*
1a	$-CH_3$	88
1b	$-CH_2CH_3$	75
1c	$-(CH_2)_2CH_3$	76
1d	$-C(CH_3)_3$	90
1e	$-(CH_2)_3CH_3$	64
1f	$-(CH_2)_5CH_3$	50
1g	$-(CH_2)_7CH_3$	61
1h	$-(CH_2)_8CH_3$	45
1i	$-(CH_2)_{10}CH_3$	66
1j	$-(CH_2)_{12}CH_3$	65
1k	$-(CH_2)_{14}CH_3$	58
1l	$-CH_2C_6H_5$	50

[a]*Nicolas Dellus, Master 1 – Juillet 2006 ; Nichola Murphy, Projet d'Erasmus, Octobre – Décembre 2006 ; Julie Vaysse, Master 1 – Juin – Juillet 2007*

2.3 Synthèse des dérivés 7-(4-(2-oxoéthylalcanoate)pipérazin-1-yl) de la CIP

Pour préparer la deuxième série des dérivés "monomères", une acylation de la CIP par le chlorure du 2-chloroéthanoyle en présence de triéthylamine dans CH_2Cl_2 conduit à la *N*-chloroacétylciprofloxacine **2** avec un rendement de 73% après purification.

La substitution nucléophile de **2** par des acides alcanoïques commerciaux a été réalisée en présence de K_2CO_3 dans le diméthylformamide (DMF) pour conduire aux dérivés 7-(4-(oxoéthylalcanoate)pipérazin-1-yl) **3a-j** (appelés "mono-oxoéthylesters") avec des rendements isolés variant de 27 à 75% (**Schéma 2**, **Tableau 42**).

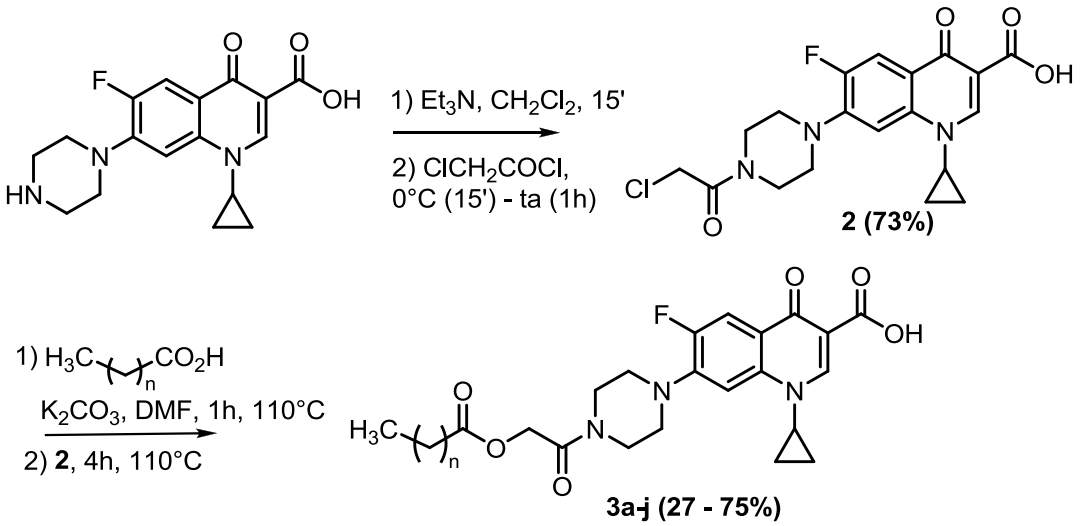


Schéma 2 Préparation des dérivés 7-(4-(oxoéthylalcanoate)pipérazin-1-yl) de la CIP **3a-j**

Tableau 42 Rendements de synthèse des dérivés 7-(4-(oxoéthylalcanoate)pipérazin-1-yl) de la CIP **3a-j** "mono-oxoéthylesters"[a]

Comoposé	*n*	*Rdt, %*
3a	0	60
3b	2	63
3c	4	75
3d	6	45
3e	7	27
3f	8	47
3g	9	43
3h	10	46
3i	12	41
3j	14	46

[a] *Florence Argeliès, Master 2, Février – Juin 2006.*

L'hydrolyse enzymatique ou chimique de ces dérivés **3a-j** conduirait à la formation de la CIP native ou du dérivé 7-(4-(2-hydroxyéthanoyle)pipérazin-1-yl) **4** (**Figure 25**).

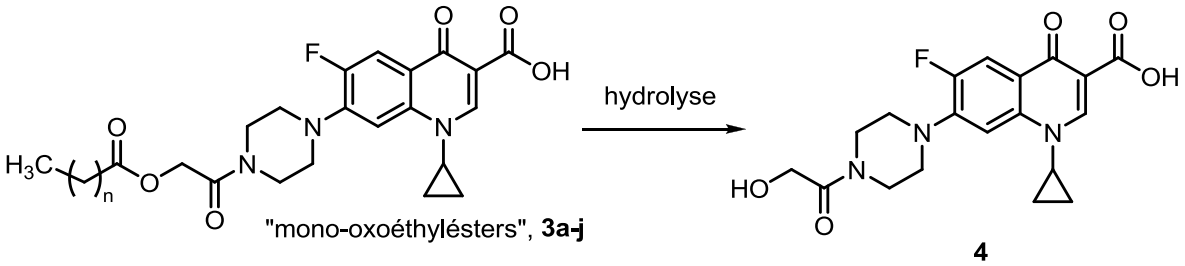


Figure 25 Hydrolyse possible des dérivés "mono-oxoéthylesters" de la CIP

Afin d'évaluer la stabilité des composés synthétisés **3a-j** et de pouvoir comparer leurs activités biologiques à celles du produit d'hydrolyse, le composé **4** a été synthétisé. L'acylation de la CIP par le chlorure de benzyloxyéthanoyle dans CH_2Cl_2 conduit au dérivé 7-(4-(2-benzyloxyéthanoyle)pipérazin-1-yl) **5** (rdt: 43%) qui, soumis à une hydrogénation catalytique (Pd/C) dans le DMF, permet d'obtenir le composé **4** avec un rendement de 60% (**Schéma 3**).

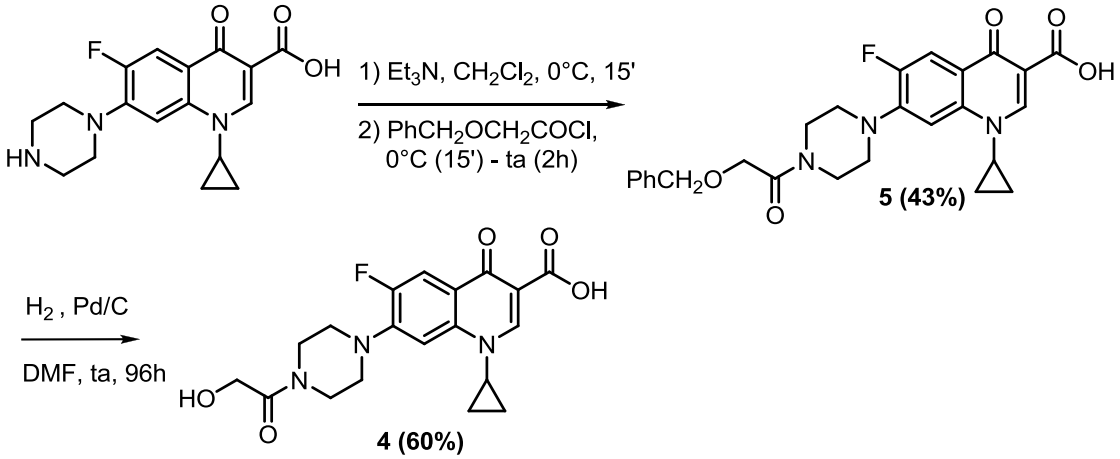


Schéma 3 Synthèse du dérivé 7-(4-(2-hydroxyéthanoyl)pipérazin-1-yl) de la CIP **4** *(Florence Argeliès, Master 2, Février – Juin 2006)*

2.4 Synthèse des dérivés 7-(4-(alkyloxycarbonyl)pipérazin-1-yl) de la CIP

La troisième série des dérivés 7-(4-(alkyloxycarbonyl)pipérazin-1-yl) de la CIP (nommés "mono-carbamates") a été préparée par condensation de la CIP avec divers alkylchlorofomates commerciaux. La réaction a été réalisée dans le CH_2Cl_2 et les composés **6a-f** ont été isolés avec des rendements allant de 33% à 66% (**Schéma 4**, **Tableau 43**) Seul le composé **6g** portant un groupement *t*-butyloxycarbonyle (Boc) a été préparée en utilisant Boc_2O dans un mélange hydroxyde de sodium aqueux 2M / dioxane (**Schéma 4**, **Tableau 43**).

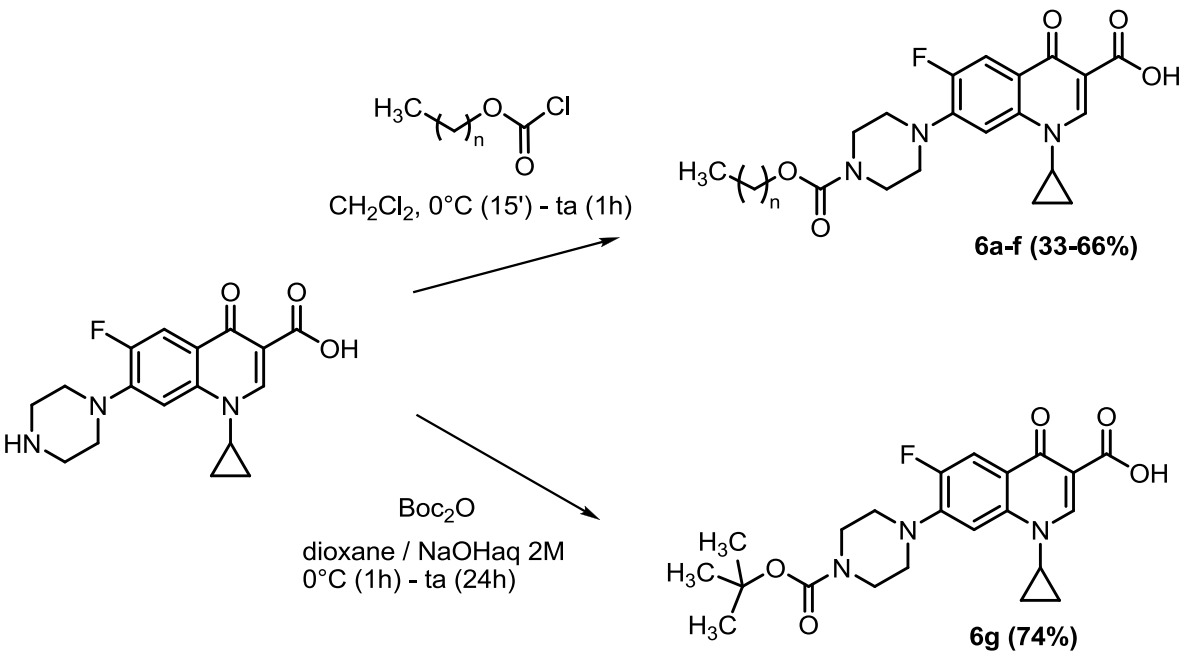


Schéma 4 Synthèse des dérivés 7-(4-(alkyloxycarbonyl)pipérazin-1-yl) de la CIP **6a-g**

Tableau 43 Rendements de synthèse des dérivés 7-(4-(alkyloxycarbonyl)pipérazin-1-yl) de la CIP **6a-g**: "mono-carbamates"[a]

Composé	*n*	*Rdt, %*
6a	1	66
6b	3	42
6c	7	37
6d	8	33
6e	9	41
6f	11	38
6g	-	74

[a] *Nichola Murphy, Projet d'Erasmus, Octobre – Décembre 2006 ; Julie Vaysse, Master 1 – Juin – Juillet 2007.*

Les dérivés "monomères" de la CIP appartenant à ces trois séries homologues ont été évalués pour leurs activités antibactériennes, antiprolifératives et antimycobactériennes. Les résultats biologiques obtenus, qui seront discutés dans le chapitre suivant, nous ont conduit à explorer la synthèse de dérivés "dimères" bis-C-7-((4-substitué)pipérazin-1-yl) de la CIP. Les travaux préliminaires de synthèse avaient déjà été réalisés au début de cette thèse, qui a été concentrée sur l'optimisation de la synthèse de ces dérivés dimères de CIP.

3. “Dimeric” ciprofloxacin derivatives

3.1 Choice of compounds to develop

We were motivated to develop a series of C-7-((4-substituted)piperazin-1-yl)-linked CIP “dimers” because of several practical and theoretical considerations.

The influence of lipophilicity on antibacterial activity was extensively studied and it was proven to have a positive effect on activity against Gram-positive microorganisms. This feature is thought to be related with increased penetration of more lipophilic compounds through the single cellular wall. Inspired by biological results obtained for previously synthesized “monomeric” CIP derivatives, that had an enhanced activity against standard and resistant *S. aureus* strains (which will be discussed in the corresponding 0), we extended our field to “dimeric” compounds as more lipophilic agents.

Increased steric bulk was proven to enhance the activity towards strains of *S. aureus* with efflux-mediated resistance. In fact, multidrug-efflux proteins are less susceptible to bulkier and lipophilic agents. This could be responsible for the enhanced activity of “monomeric” CIP derivatives. Thus bulkier and more lipophilic “dimers” should be interesting candidates for their evaluation against resistant strains of *S. aureus*.

It has been long proposed that more than one molecule of quinolone binds to the target enzyme; however until recently there was no clear evidence about the structure of binding complex. Nowadays, it is thought that the target bacterial enzyme forms a ternary complex with DNA and two molecules of quinolone which are oriented in a “tail-to-tail” manner (**Figure 26**).

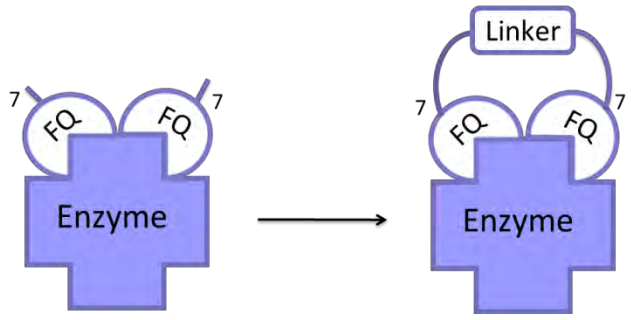
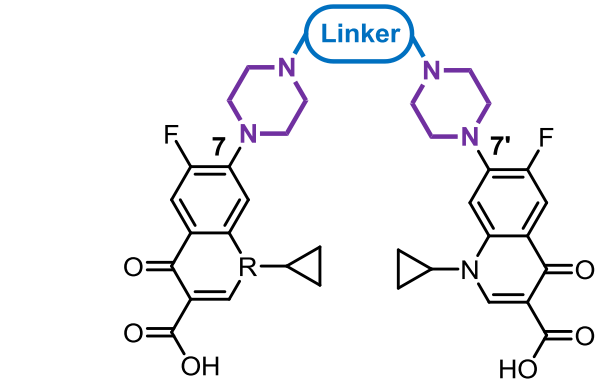


Figure 26 Schematic view of quinolone-enzyme complex and representation of multibinding concept (7 – piperazinyl “tails” of quinolone)

This finding prompted the detailed study of quinolone “dimers” with most representative works of *Kerns et al* (**Figure 27**). The authors demonstrated increased activity of “dimeric” compounds towards *S. aureus* strains in comparison to parent FQs. However linkers used by *Kerns* were relatively rigid and short. Taking into account recent structural data on the ternary

quinolone-enzyme-DNA complex, these “dimers” were unlikely to have a favorable structure to allow multiple binding.



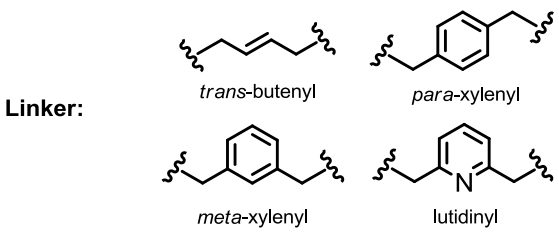


Figure 27 C-7-((4-substituted)piperazin-1-yl)-linked CIP “dimers” developed by *Kerns et al*

The situation with anticancer activity is more complicated because of multiple mechanisms of action and multiple targets for quinolones. There are less data in the literature on SAR studies for antiproliferative quinolones, in comparison with antibacterial ones. Expanding this relatively unexplored area could reveal important dependencies and trends.

For all these reasons, we decided to synthesize two series of CIP “dimers”. We chose a flexible linker based on alkyl chain of varying length. The linking groups were chosen as the ones from the most active “monomeric” derivatives, that were “ester” and “amide” functions (**Figure 28**).

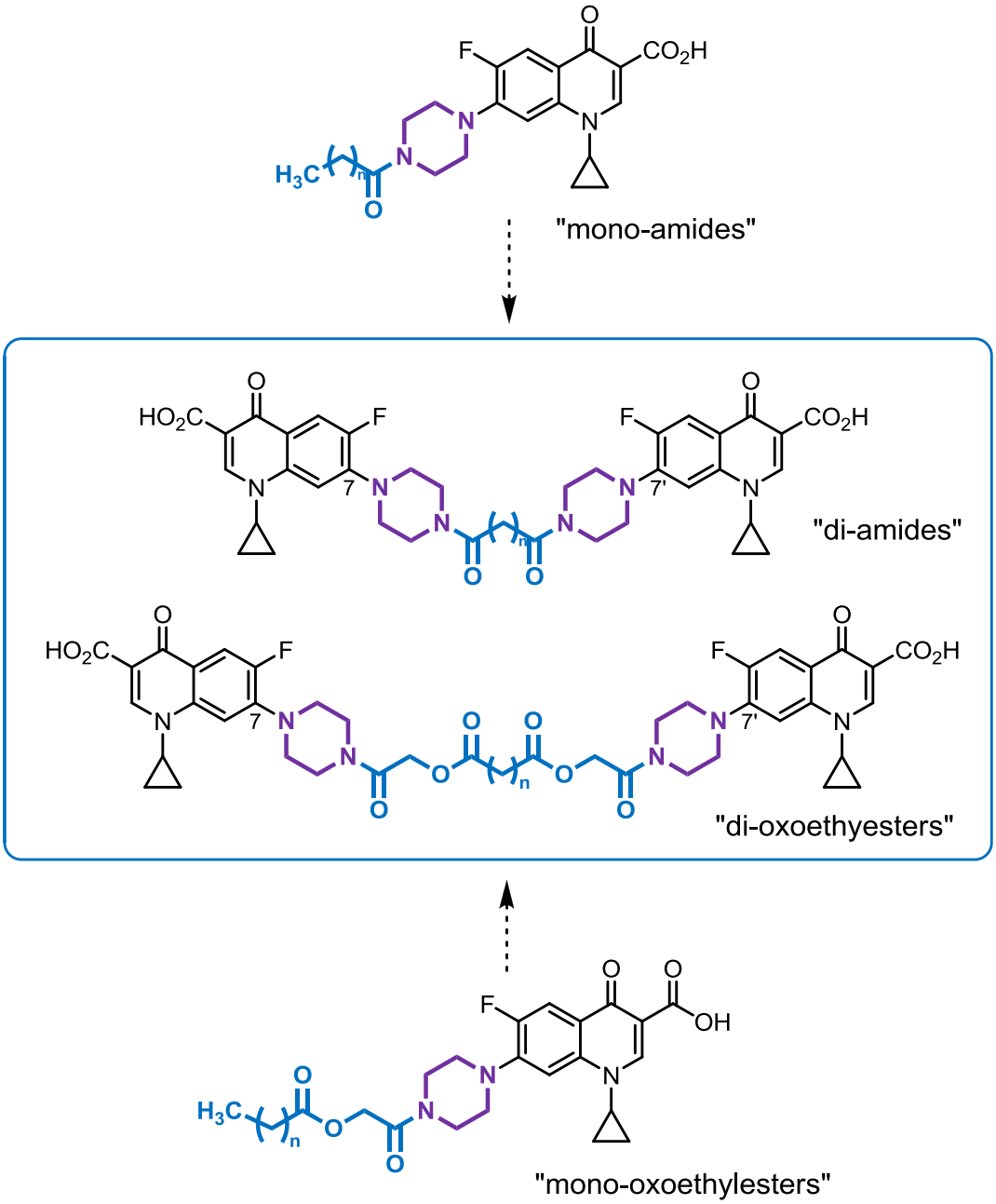


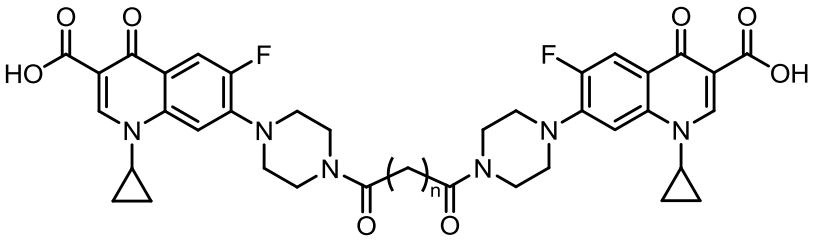
Figure 28 The choice of C-7-((4-substituted)piperazin-1-yl)-linked CIP "dimers"

In summary, the rationale for the introduction of a carbon-chain spacer between two CIP moieties is based on the binding of two quinolone molecules per target bacterial enzyme[27] and successful "dimers" examples in the literature. Varying the bridge length should also result in compounds with different hydrophobicity and steric hindrance, thus providing a useful model for antibacterial and antiproliferative SAR studies. In the next section, we will discuss the synthetic pathway for C-7-((4-substituted)piperazin-1-yl)-linked CIP "dimers" and some challenges faced.

3.2 Synthesis of 7-(4-(alkanoyl)piperazin-1-yl))-linked CIP "dimers"

3.2.1 Previous attempts

There are a lot of classical methods described in the literature for the synthesis of carboxamides. First of all we searched through the most suitable and effective procedure to access to 7-(4-(alkanoyl)piperazin)-linked CIP "dimer" **7a-f**, termed "di-amides" (**Figure 29**).

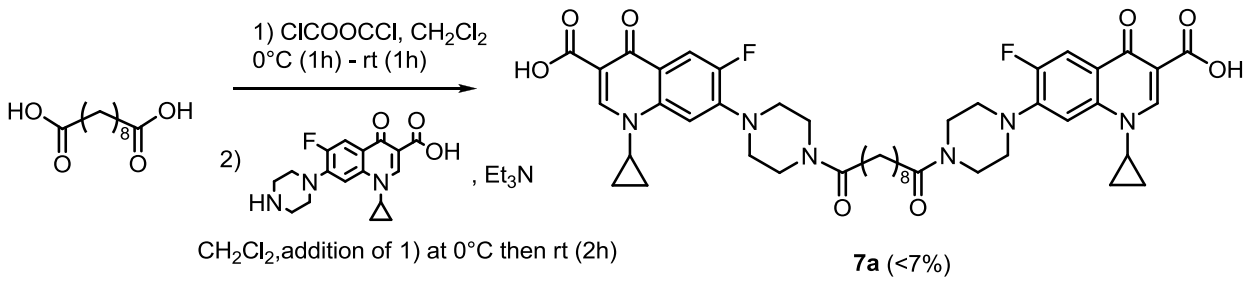


7a-f n = 8, 10, 12, 13, 14, 16

Figure 29 Target molecules of 7-(4-(alkanoyl)piperazin)-linked CIP "dimers"

Preliminary assays were done to access to the 7-(4-(octanoyl)piperazin)-linked CIP "dimer" **7a** (n=8). Acylation of aliphatic amines with acyl chlorides in the presence of triethylamine is a general and reliable method for the carboxamides synthesis. This method applied to the acylation of CIP by commercially acyl chlorides led to the "mono-amide" series **1a-l** in good yields (**Tableau 41**). Nevertheless, if decanedioyl chloride and dodecanedioyl chloride were commercially available, the other diacyl chlorides (n = 12, 13, 14 and 16) were not available. So, we decided to generate *in situ* the diacyl chloride by a classic reaction of oxalyl chloride with commercially available alkanedioïc acids.

After the stepwise procedure involving initial reaction of oxalyl chloride with decanedioïc acid in CH_2Cl_2 and the subsequent addition to a solution of CIP and triethylamine, the isolated yield of the target "di-amide" **7a** was less than 7% (**Scheme 5**). The possible explanation for the observed yield is that FQ heterocyclic system doesn't tolerate strong acidic environment generated by commonly used thionyl and oxalyl chlorides.



Scheme 5 Synthesis of 7-(4-(octanoyl)piperazin)-linked CIP "dimer" **7a** through an acyl chloride pathway

During further attempts the main idea was to utilize a "powerful" activating agent for alkanoïc diacid which is mild enough to preserve the heterocyclic system of CIP. It was decided to avoid acidic agents by using carbodiimide reagents which are a widely used class of agents for peptide synthesis. Dicyclohexyl carbodiimide[163] (DCC) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride salt[164] (EDCI) are frequently used as a carboxyl activating agent for amide bond formation. Additives, such as *N*-hydroxybenzotriazole (HOBt), *N*-hydroxysuccinimide (NHS) or 2-thiazoline-2-thiol (2-TT) are often added to increase yields and/or decrease side reactions (**Figure 30**).

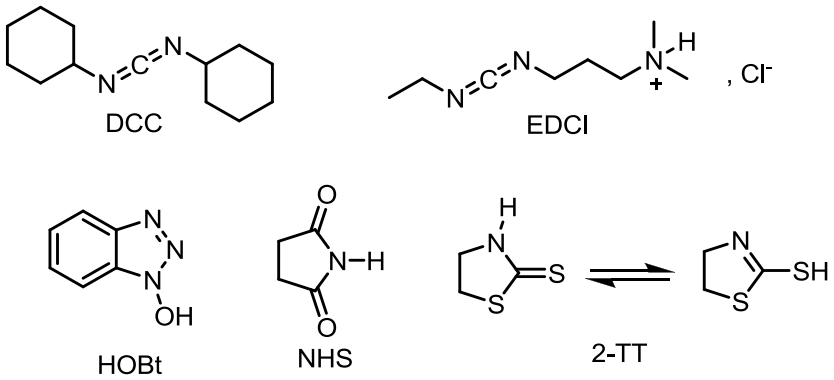


Figure 30 Examples of carbodiimide reagents and additives

No additional amine is theoretically required during this one-pot procedure. Concerning the mode of reaction (**Figure 31**), the carbodiimide reacts with the carboxylic acid to form the O-acylisourea **I**. The nucleophilic substitution of amine on **I** yields the desired amide and the urea by-product **II**. The isolation of the symmetric carboxylic anhydride from the reaction mixture suggested, however, that a more complex mechanism might co-exist[165]. The driving force of this reaction is the formation of the urea **II**. However, the racemisation and acetyl transfer which form the unreactive N-acylurea **III** are often observed This side reaction can be considerably

diminished by carrying out the procedure in a two-step manner : the initial reaction between the acid and the coupling agent is carried out at 0°C, followed by amine addition. Furthermore, a selected nucleophile can be used as an additive. The nucleophile also prevents the undesired product formation, because it reacts faster than the competing acyl transfer and generates the intermediate **IV** on which the nucleophilic substitution by the amine yields the desired amide.

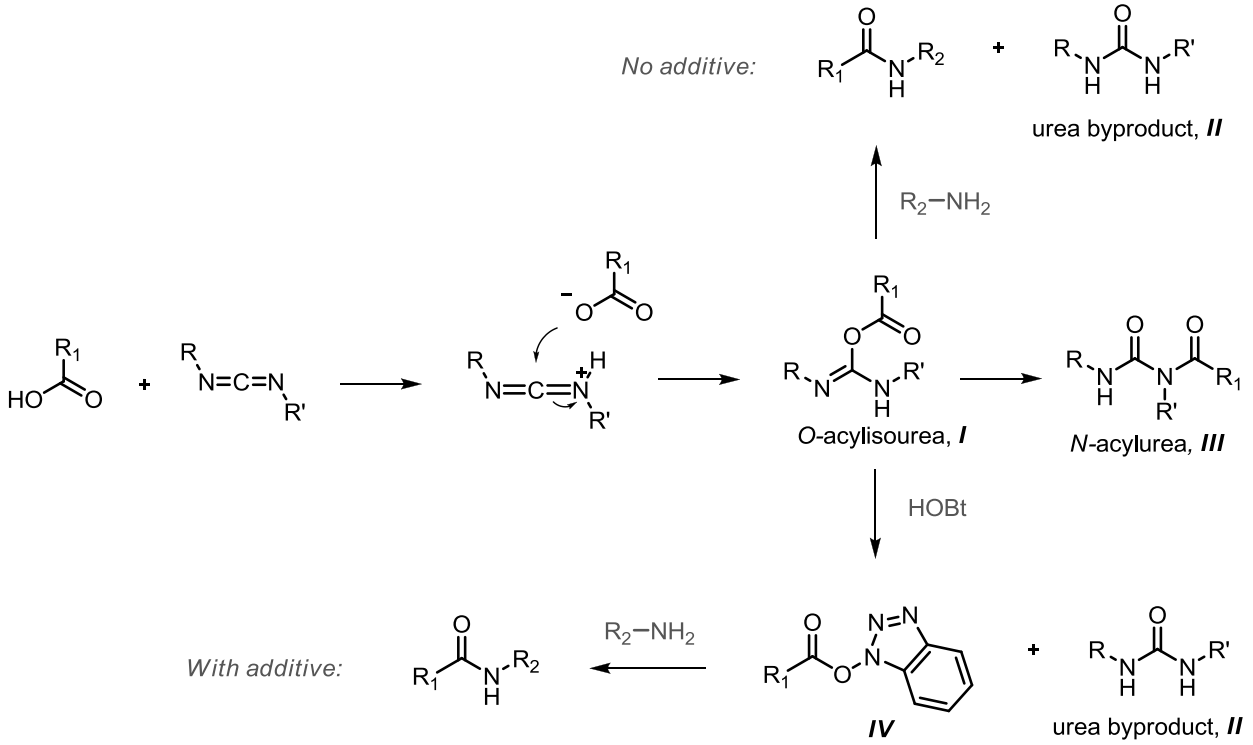


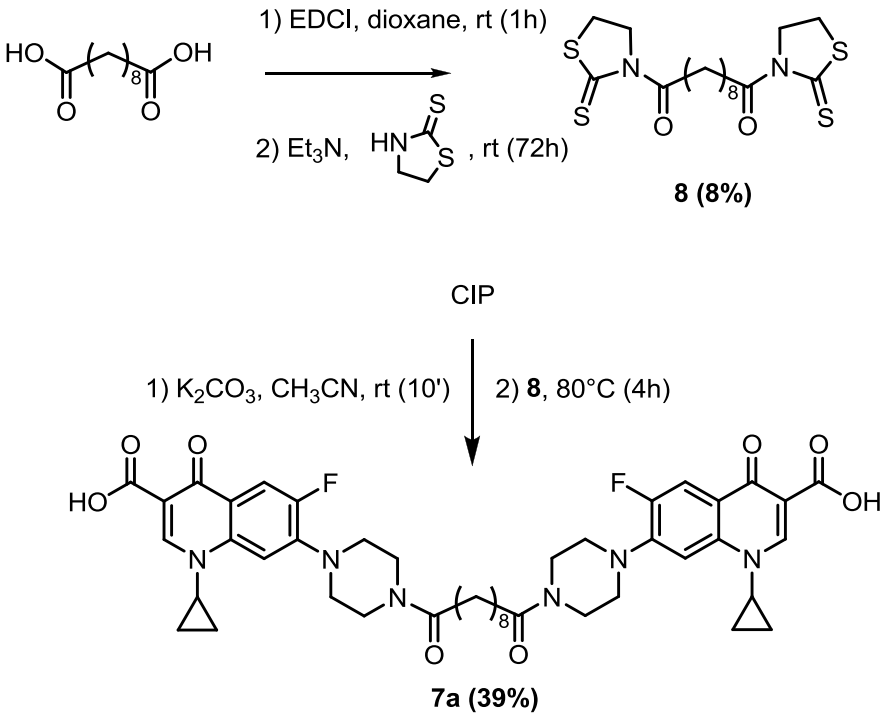
Figure 31 Carbodiimide-assisted amide synthesis, with and without additive (HOBt is showed as example of nucleophile)

An attempt was conducted by using the DCC / 2-TT system in dioxane. However, as the desired "di-amide" **7a** and the resulting dicyclohexylurea byproduct showed similar low solubility in common solvents, it was too difficult to separate them.

This practical problem was solved by the use of EDCI / 2-TT system. The resulting urea was water-soluble and thereby could be easily removed by extractive work-up.

In this third attempt, 1,10-bis(2-thioxothiazolidin-3-yl)decane-1,10-dione **8** was obtained by coupling 1,10-decanedioïc acid with 2-TT in the presence of EDCI in dioxane. Unfortunately, due to tedious purifications, the yield was very low (8%). The "di-amide" **7a** was synthesized by the subsequent addition of **8** to a solution of CIP and K_2CO_3 in acetonitrile (CH_3CN) in 39% yield. However, the overall yield after these two steps was very low 3% (**Scheme 6**). This result

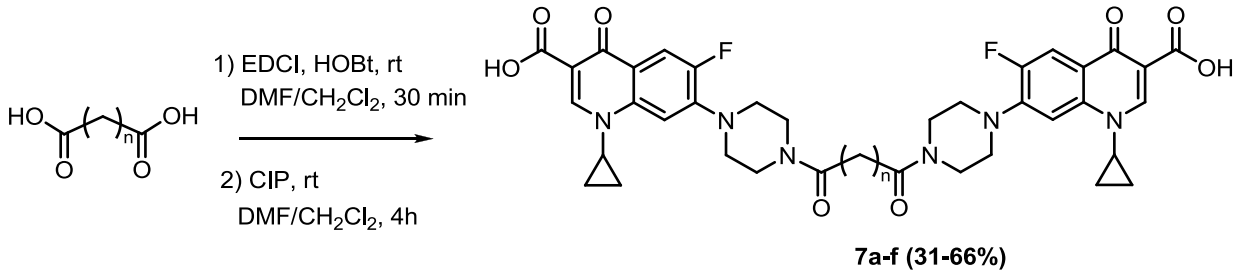
is apparently due to the wrong choice of the 2-TT in the first step, leading to an intermediate **8** hard to purify.



Scheme 6 Synthesis of CIP "di-amide" **7a** through a 2-TT pathway

3.2.2 Current work

An attempt conducted by using EDCI / HOBt system was successful and the desired "di-amide" **7a** was easily isolated in 48% yield. This synthetic pathway was then applied to the synthesis of the target CIP "di-amides" **7a-f** (**Scheme 7, Table 44**). Initial activation of α,ω-alkanedioïc acid (1 equivalent) was performed by reacting with EDCI (2.5 equivalents) and HOBt (2.5 equivalents) in 1:1 DMF / CH_2Cl_2 mixture for 30 minutes at room temperature. The subsequent addition of CIP (2.3 equivalents) and stirring for 4 hours at room temperature, was followed by a precipitation of the compound and water washing. The purification by column chromatography on silica provided the corresponding 7-(4-(alkanoyl)piperazin-1-yl))-linked CIP "dimers" **7a-f** in yields ranging from 31 to 66%. Certain derivatives of this series were difficult to purify which explains differences between yields.



Scheme 7 Preparation of 4-(alkanoyl)piperazin-1-yl-linked CIP "dimers" **7a-f**

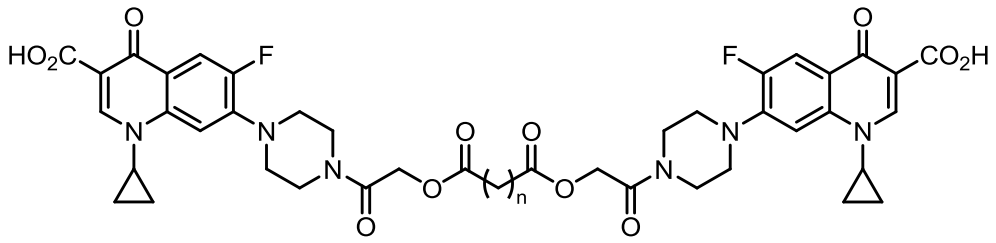
Table 44 Yields of CIP "di-amides" derivatives **7a-f**

Compound	*n*	*Yield, %*
7a	8	48
7b	10	31
7c	12	66
7d	13	61
7e	14	55
7f	16	53

In summary, several synthetic methods have been used during our attempts to synthesize CIP "di-amides" derivatives. We have faced degradation-related problems using oxalyl chloride pathway whereas challenging purification was a major issue when DCC or EDCI / 2-TT systems were used. As a result, EDCI / HOBt system was found to be the most efficient in terms of yields and practical convenience. Having successfully synthesized the "di-amide" series of CIP derivatives we have shifted to the "di-oxoethylester" series which will be discussed in greater details in the next section.

3.3 Synthesis of 7-(4-(oxoethylalkanoate)piperazin-1-yl)-linked CIP "dimers"

Keeping in mind that we wanted to establish a SAR of synthetized FQs derivatives, the length of the alkyl chain was preserved as before that for "di-amides" series (**Figure 32**)



9a-f n = 8, 10, 12, 13, 14, 16

Figure 32 Target molecules of 7-(4-(oxoethylalkanoate)piperazin-1-yl)-linked CIP "dimers"

The 7-(4-(oxoethylalkanoate)piperazin-1-yl)-linked CIP "dimers" **9a-f**, termed "di-oxoethylester" derivatives were prepared by using the method previously described for "mono-oxoethylester" analogues **3a-j** (**Schéma 2**, **Tableau 42**). From the synthetic point of view it consists of two classical nucleophilic substitution reactions.

First step was the substitution of chloroacetyl chloride by the 4-(piperazinyl)nitrogen of CIP to afford 7-(4-(2-chloroacetyl)piperazin-1-yl) CIP derivative **2** as described previously in **Schéma 2**.

The next stage was the condensation of carboxylic diacids with derivative **2**. The mechanism involves classical nucleophilic attack of the *in situ* generated dicarboxylate anion on the derivative **2** (**Figure 33**). This step was a subject for condition optimization during preliminary studies which were carried out in our group.

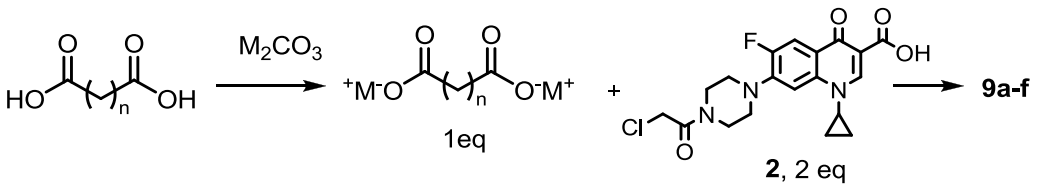


Figure 33 Schematic representation for the preparation of CIP "di-oxoethylesters"

3.3.1 Optimisation of reaction conditions for 1,12-dodecanoïc diacid

In order to find the best conditions for the condensation of carboxylic diacids, it was decided to perform a series of optimization experiments conducted by using 1,12-dodecanedioïc acid (n = 10) to produce **9b** derivative (**Figure 32**).

All optimization runs involved the following typical procedure : A solution of carboxylic diacids (1 equivalent) and M_2CO_3 (2 equivalents) in DMF or CH_3CN under argon was stirred during 2 hours. Compound **2** (2 equivalents) and TBAI (none, 0.2 or 2 equivalents) were added and was stirred at the mentionned temperature for 3, 5 or 7 days (**Table 45**).

Firstly, the conditions previously described for the synthesis of "mono-oxoethylesters" **3a-j** were used, that were K_2CO_3 as a base and DMF as a solvent. However, in comparison with the "monomer" analog, these conditions gave "di-oxoethylester" derivative in lower yield (10-15% for **9b** versus 46% for **3h** (n=10)) and required longer reaction time, from 4 hours for **3h** to 5-7 days for **9b** (**Table 45, entries 1-2**).

Changing the solvent to CH_3CN allowed us to slightly decrease the reaction time from 7 days to 5 days. However, yield (15%) was still poor and additional optimization was needed. (**Table 45**, **entry 3**).

We decided to use tetrabutylammonium iodide (TBAI) in order to increase the yield of this reaction. TBAI is supposed to *in situ* convert *N*-chloroacetylciprofloxacin into the more reactive *N*-iodo-analog. Because iodides are generally more reactive in nucleophilic substitution, we expected a faster completion of the reaction. However, when a catalytic amount of TBAI (0.2 equivalent) was used, no improved yield was observed (**Table 45**, **entry 4**). Whereas a stoechiometric amount of TBAI (2 equivalents) led to an enhanced yield with a 3-fold increase from 15 to 46% and allowed us to reduce the reaction time to a 3 days period (**Table 45**, **entry 5**). However, it was noticed that the crude mixture was more complex when reaction was carried out in CH_3CN instead of DMF

It is known that cesium counterion and dipolar aprotic solvents (such as DMF, dimethylsulfoxide (DMSO) and hexamethylphosphorotriamide) facilitate nucleophilic substitution reactions. The more voluminous cesium cation tends to form weaker ionic bond with hard anion, thereby freeing nucleophilic anion from the ionic pair and making it more active. DMF, as a polar aprotic solvent, stabilizes free anion thereby promoting the dissociation of the carboxylic salt. While having similar yields, the use of cesium carbonate (Cs_2CO_3) / DMF combination (**Table 45**, **entry 6**) allowed us to decrease the reaction temperature in comparison to K_2CO_3 / CH_3CN conditions. The formation of byproducts were less important in DMF at 50°C leading to easier purifications. This effect of the temperature seemed to be important to avoid the

formation of byproducts as if the reaction was conducted in DMF at temperature above 60°C more complex mixtures were obtained.

Table 45 Optimisation runs for the synthesis of 7-(4-(2-oxoethyldodecanoate)piperazin-1-yl))-linked CIP "dimer" **9b**

Entry	*Base*	*Solvent*	*Relative amounts of TBAI*	*Temperature*	*Reaction time*	*Yield (%)*
1	K_2CO_3	DMF	-	80°C	5 days	10
2	K_2CO_3	DMF	-	80°C	7 days	15
3	K_2CO_3	CH_3CN	-	80°C	5 days	15
4	K_2CO_3	CH_3CN	TBAI (0.2eq)	80°C	5 days	15
5	K_2CO_3	CH_3CN	TBAI (2eq)	80°C	3 days	46
6	Cs_2CO_3	DMF	TBAI (2eq)	50°C	3 days	50

In summary, after a series of experiments two set of optimized reaction conditions were found (**Table 45**, **entries 5-6**). They were further applied to the other chain lengths in order to compare their effectiveness for the synthesis of both short and long-chain "dimers". These studies will be described in the next section.

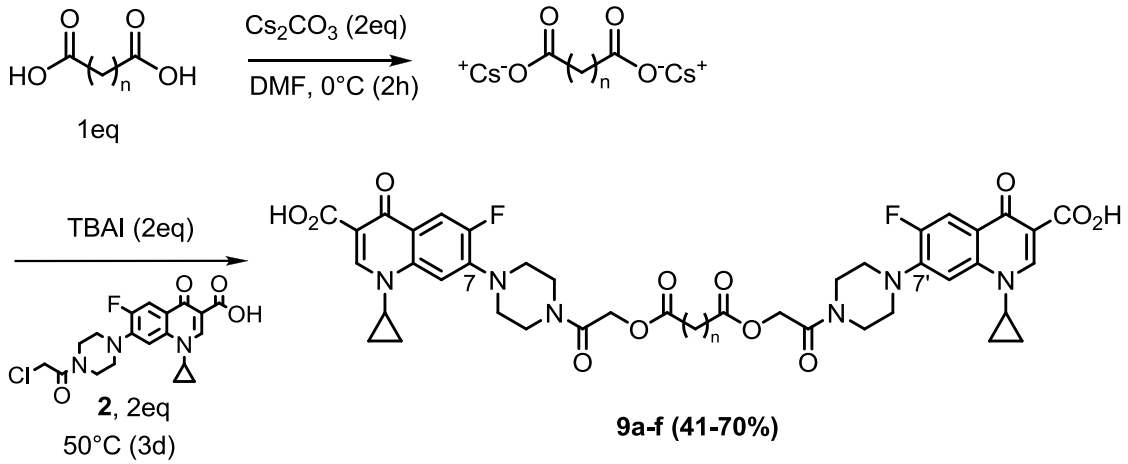
3.3.2 Application of optimized runs to the synthesis of 7-(4-(oxoethylalkanoate)piperazin)-linked CIP "dimers"

In order to compare the two selected methods according to the chain length, we have tested them from 1,12-decanedioïc, 1,14-tetradecanedioïc and 1,16-hexadecanedioïc acids. The results, summarized in **Table 46 (entries 1-6)**, showed that both methods provided the desired "di-oxoethylester" derivatives **9a,c,e** in better yields with Cs_2CO_3/DMF (42, 56 and 41% respectively) than K_2CO_3/CH_3CN (37, 29 and 15% respectively) combinations, specially for the synthesis of **9e** from 1,16-hexadecanedioïc acid**.** The best conditions were further applied to the synthesis of "di-oxoethylesters" **9d,f** from 1,15-pentadecanedioïc and 1,18-octadecanedioïc acids leading to the desired compounds in good yields of 56 and 70% respectively (**Table 46**, **entries 7-8**)

Table 46 Yields of "di-oxoethylester" CIP derivatives **9a,c-f** according to optimized experimental conditions

Entry	*Compound (n)*	*Base*	*Solvent*	*Catalyst*	*Temp.*	*Time*	*Yield (%)*
1	**9a** (8)	K_2CO_3	CH_3CN	TBAI (2eq)	80°C	3 days	37
2	**9a** (8)	Cs_2CO_3	DMF	TBAI (2eq)	50°C	3 days	42
3	**9c** (12)	K_2CO_3	CH_3CN	TBAI (2eq)	80°C	3 days	29
4	**9c** (12)	Cs_2CO_3	DMF	TBAI (2eq)	50°C	3 days	56
5	**9e** (14)	K_2CO_3	CH_3CN	TBAI (2eq)	80°C	3 days	15
6	**9e** (14)	Cs_2CO_3	DMF	TBAI (2eq)	50°C	3 days	41
7	**9d** (13)	Cs_2CO_3	DMF	TBAI (2eq)	50°C	3 days	56
8	**9f** (16)	Cs_2CO_3	DMF	TBAI (2eq)	50°C	3 days	70

Finally, the desired series of "di-oxoethylester" CIP derivatives **9a-f** were synthesized by the following procedure: A solution of α,ω-alkanedioïc acid (1 equivalent) and Cs_2CO_3 (2 equivalents) was stirred in DMF at 50˚C for 2 hours to obtain the corresponding carboxylate salt. Then, TBAI (2 equivalents) and *N*-chloroacetylciprofloxacin **2** (2 equivalents) were added and the reaction mixture was stirred for 3 days at 50°C. After work-up and purification by silica gel chromatography desired "di-oxoethylester" CIP derivatives **9a-f** were isolated in 41-70 % yields (**Scheme 8, Table 46, entries 2, 4, 6-8**). Yields varied because certain products were difficult to purify.



Scheme 8 Preparation of 7-(4-(oxoethylalkanoate)piperazin-1-yl))-linked CIP "dimers" **9a-f**

In summary, we have synthesized the second series of CIP "dimer" derivatives. During the optimization process we have found that, among tested combinations, the Cs_2CO_3 in DMF

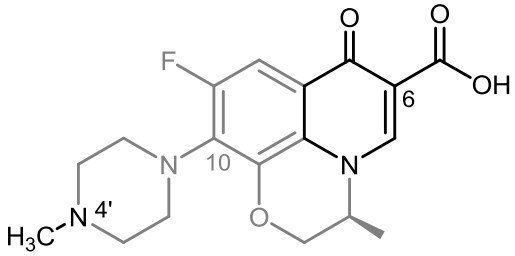
provides the highest yields for different chain lengths. Having successfully synthesized C-7-linked CIP “dimers”, we have shifted our attention to C-6 modified LEV derivatives. The detailed data concerning the synthesis of different LEV derivatives will be discussed in the next section.

4. Levofloxacin derivatives

4.1 Choice of compounds to develop

During the literature overview we have shown that the C-3 position on FQs (C-6 in LEV) bears a unique type of substituent which stands out among other positions. From the antibacterial point of view, this position has a very low tolerance for structural modifications. To date, carboxyl group is the only successful substituent present in currently marketed antibacterial quinolones. For antibacterial activity, the carboxyl group plays essential role in drug-enzyme interaction and is thought to form multiple hydrogen bonds with a quinolone binding pocket[27]. However, concerning the antiproliferative activity, some other substituents are tolerated.

LEV, as well as CIP, is highly active against wide range of gram-positive and gram-negative pathogens and is used as a second line drug for the treatment of tuberculosis. LEV also possesses some antiproliferative activity against human cancer cell lines. From the chemical point of view, the methylated nature of the *N*-4' nitrogen of C-10-piperazine moiety of LEV permits chemical transformations at carboxylic group (**Figure 34**). These properties make LEV an interesting molecule for the synthesis of C-6 modified derivatives.



Levofloxacin

Figure 34 Structure of LEV

As the upper portion of the molecule which includes the carboxy- and keto moieties is required for interactions with DNA bases, they are generally considered as essential and thus explains low tolerance for modifications at this point. In order to maintain significant degree of activity, the C-6 group of LEV should comprise structural elements with hydrogen atom able to mimic carboxyl group. Literature examples include arylamides with some representatives being equipotent to LEV towards *S. aureus*[85]. The amide moiety bears additional hydrogen atom which could form hydrogen bonds with enzyme residues. The bonding could be formed with one or both tautomeric forms of the amide (**Figure 35**).

In contrast to antibacterial activity, antiproliferative potency tolerates structural changes at C-3 carboxylic acid of quinolones with a number of successful examples.

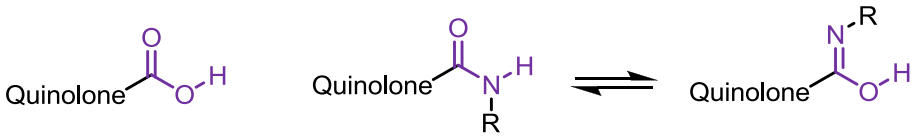


Figure 35 Carboxyl group mimicking by amide group

Taking into account these considerations we synthesized series of alkyl carboxamides (termed "mono-amides") and "dimeric" analogs (termed "di-amides") of LEV. We have also decided to synthesize a series of acyloxymethyl esters of LEV that would represent prodrugs of LEV. In the literature various acyloxyalkyl ester prodrugs of butyric acid with anticancer activity were described.[166].

In a similar way to the derivatives synthesized from CIP, varying the chain length should result in compounds with different hydrophobicity and steric hindrance, thus providing a useful model for SAR studies.

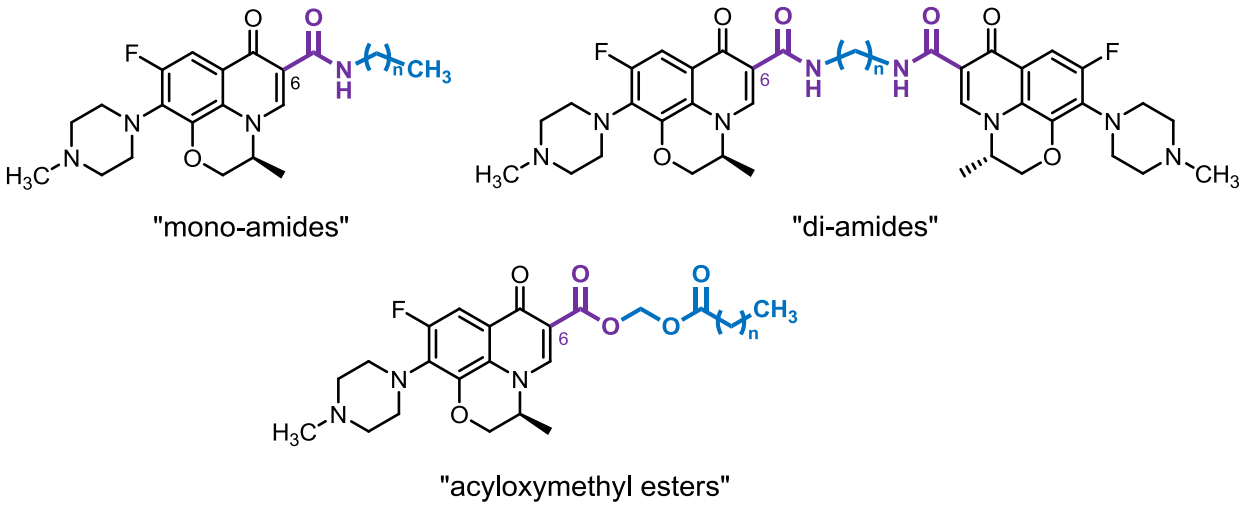


Figure 36 Target molecules of LEV derivatives**.**

In summary, after reviewing the literature and taking into account previous results of our group we have decided to synthesize two types of LEV derivatives: carboxamides and acyloxymethyl esters (**Figure 36**). The detailed synthetic pathway and challenges faced will be described in the next section.

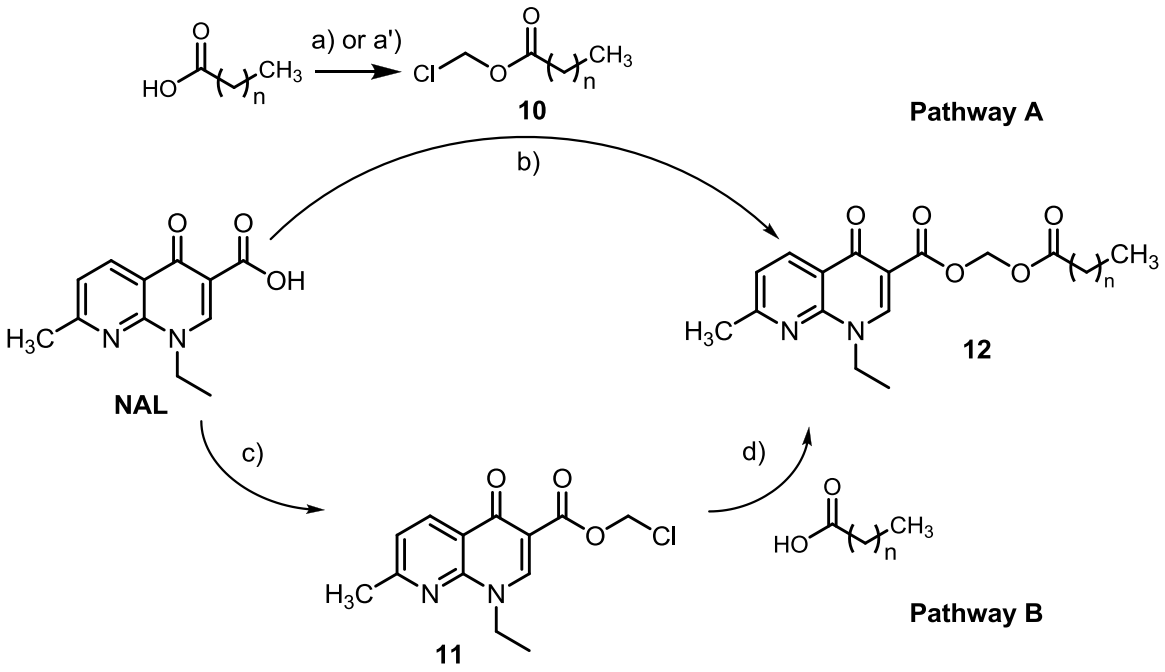
4.2 Synthesis of C-6-acyloxymethyl esters of LEV: Previous attempts

4.2.1 Synthesis of C-3-acyloxymethyl esters of nalidixic acid

The FQ modification at C-3 position was first carried out in our group by using NAL, a parent compound for the quinolone family[167]. Two possible pathways were evaluated for the synthesis of acyloxymethyl esters of NAL (**Scheme 9**). The pathway **A** consists in a nucleophilic substitution of chloromethyl alkanoates **10**, synthesized from alkanoic acids, by NAL carboxylate. The first step of the second pathway **B** consists in synthesis of the chloromethyl ester of NAL **11** and in the subsequent nucleophilic substitution by various fatty carboxylates (**Scheme 9**).

Two methods of chloromethylation of alkanoic acids were employed, either by a phase-transfert system or by the use of a Lewis acid in presence of 1,3,5-trioxane (pathway **A**). It was shown that that higher yields of alkylcarbonyloxymethyl chlorides **10** could be obtained by the phase-transfert system. Unfortunately, when attempting the alkylation of NAL potassium salt with one equivalent of these reagents in DMF at 50°C for 24 h, complex mixtures were obtained resulting from incomplete reaction (even in the presence of sodium iodide) or decomposition. The corresponding acyloxymethyl esters **12a-b** were then isolated in poor yields ranging from 22% to 35% (**Table 47**).

On the contrary, the pathway **B** provided the clean chloromethylation of NAL by using chloromethyl chlorosulfate ($ClCH_2OSO_2Cl$) in a phase-transfer H_2O/CH_2Cl_2 system. The chloromethyl NAL derivative **11** was obtained in a 55% yield. Further condensation with the salt of fatty acid in DMF at room temperature for 4 days rendered the corresponding acyloxymethyl esters **12a-d** in 50-70% yields (**Table 47**).



Reagents and conditions : **Pathway A** : a) i – $CH_3(CH_2)_nCOOH$, Na_2CO_3, H_2O (rt or 100°C according to n) ii – CH_2Cl_2, $(nBu)_4NHSO_4$, ClO_2SOCH_2Cl, 0°C (1h) – rt (18h) **or** a') i – ClCOCOCl, CH_2Cl_2, 0°C (1h) – rt (2h), ii- $ZnCl_2$, $CaCl_2$, rt (15') iii – 1,3,5-trioxane, 0°C (2h) – rt (20h). b) **10**, K_2CO_3, DMF, 50°C (24h). **Pathway B** : c) i – Na_2CO_3, H_2O, rt (90') ii – CH_2Cl_2, $(nBu)_4NHSO_4$, ClO_2SOCH_2Cl, 0°C (1h) – rt (18h). d) i – $CH_3(CH_2)_nCOOH$, K_2CO_3, DMF, 50°C (5h) ii – **11**, rt (4d)

Scheme 9 Preparation of acyloxymethyl esters of NAL

Table 47 Yields (%) of acyloxymethyl esters of NAL according to pathways **A** or **B**

Compound	*n*	*Pathway **A** (from **10**)*	*Pathway **B** (from **11**)*
12a	6	35	70
12b	10	22	50
12c	12	-	60
12d	14	-	56

The pathway **B** turned out to be more convenient in terms of purification and yields and above all it does not require the synthesis of each chloromethyl alkanoates **10**. So, this pathway **B** was chosen for the preparation of a series of acyloxymethyl esters of LEV.

4.2.2 Synthesis of acyloxymethyl esters of LEV

However when this method **B** was applied to LEV the first stage couldn't be completed. The two alternative protocols for the chloromethylation were tried, but none of them provided the desired chloromethyl ester of LEV **13** (**Figure 37**).

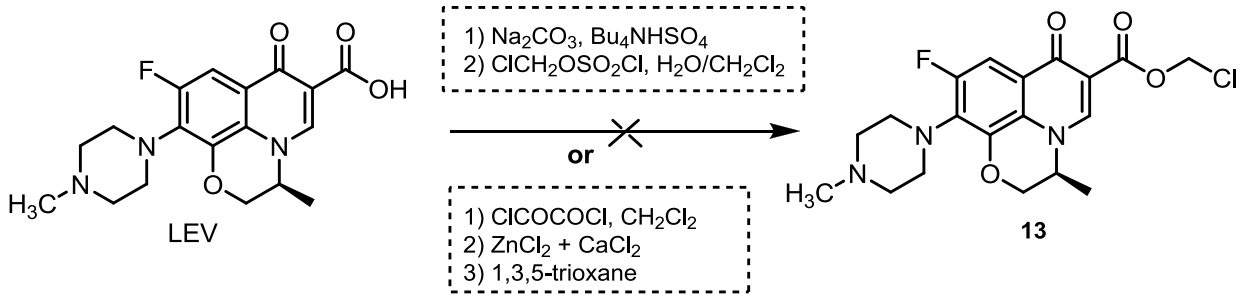


Figure 37 Unsuccessful attempts for the chloromethylation of LEV

So, it was decided to use the pathway **A** for the synthesis of acyloxymethyl esters of LEV **14**, that is the direct alkylation of LEV with various chloromethyl alkanoates (**Figure 38**). In order to apply this method to various alkyl chain lengths it was necessary to synthesize a series of alkylcarbonyloxymethyl chlorides **10**, except for the commercially chloromethyl butyrate (n = 2) **10a**.

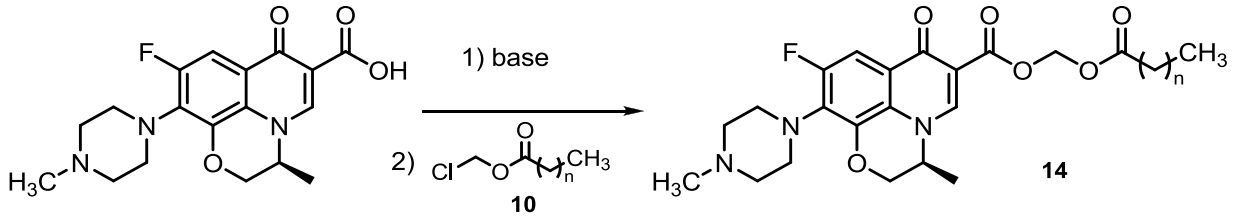
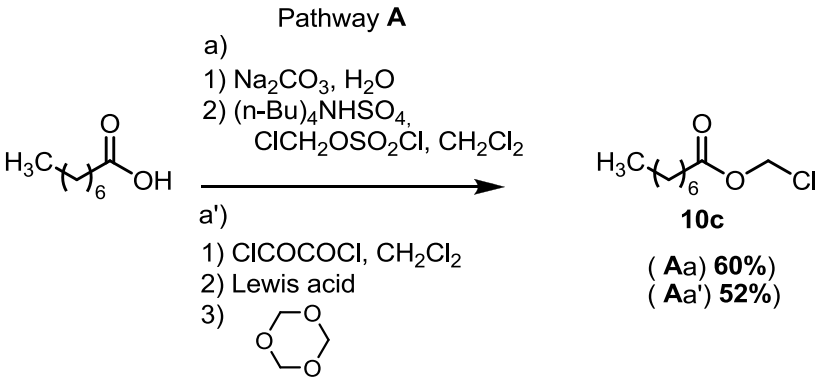


Figure 38 Schematic representation of the preparation of acyloxymethyl esters of LEV **14**

4.2.3 Synthesis of chloromethyl alkanoates

Examination of the literature on the preparation of chloromethyl esters revealed two main protocols : alkylation of carboxylates with chloromethyl chlorosulfate in phase-transfer conditions[168] (**Scheme 9,** pathway **A** a)) and condensation of an aldehyde with acid chlorides in the presence of a Lewis acid[169] (**Scheme 9,** pathway **A** a')). Preliminary studies involving the synthesis of chloromethyl octanoate **10c** (n=6) revealed that the **A** a) method provides higher

yields (60% yield for **10c**) but uses hazardous, potentially carcinogenic alkylating agent, that is chloromethyl chlorosulfate. In order to improve the **A** a') procedure, several Lewis acids were tried for the preparation of chloromethyl octanoate **10c** (**Scheme 10**, **Table 48**). It was observed that the use of zinc chloride in the presence of calcium chloride improved the yield of reaction to 52%.



Scheme 10 Procedures for the synthesis of chloromethyloctanoate **10**c

Table 48 Optimization of the synthesis of chloromethyl octanoate (n = 6) [167]

Pathway **A**	*Lewis Acid*	*Yield of **10c**, %*
a)	–	60[a]
a')	$SnCl_4$	33[b]
	$ZrCl_4$	9[b]
	$AlCl_3$	16[b]
	$ZnCl_2$	25[b]
	$ZnCl_2 + CaCl_2$	57[b] (52[a])

[a]Yields after isolation. [b]Yields calculated from ^{1}H NMR (Nuclear Magnetic Resonance) spectra of mixtures obtained after usual workup.

After these optimization runs with chloromethyl octanoate, both methods were applied to the synthesis of a series of lipophilic alkylating agents. It was observed that the pathway **A** a) is often more effective for the synthesis of chloromethyl alkanoate in comparison with the pathway **A** a') (**Table 49**).

Table 49 Comparative results for the **s**ynthesis of chloromethyl alkanoates

Compound	*n*	*Yield, %*	
		*Pathway **A** a)*	*Pathway **A** a')*
10b	4	68%	–
10c	6	60	52
10d	8	77, 87[a]	–
10e	10	84, 87[a]	41
10f	12	82	–
10g	14	80	56, 69[a]
10h	16	–	58, 72[a]
10i	18	81	–

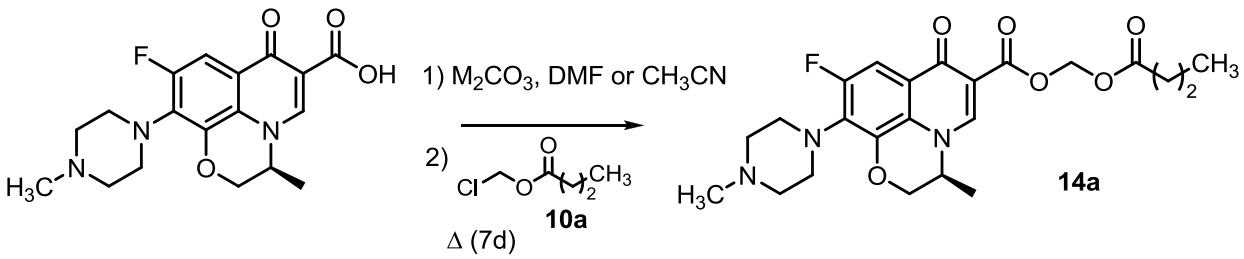
[a]Yields from multiple experiments

In summary, preliminary assays have shown that the pathway **B** (**Scheme 9**) derivatization worked well with NAL but failed when it was applied to LEV. Alternative **A** pathway comprising the LEV derivatization with various chloromethyl alkanoates was promising. Therefore, a series of these lipophilic alkylating esters was prepared for the synthesis of various acyloxymethyl esters of LEV.

4.3 Synthesis of C-6-acyloxymethyl esters of LEV: Optimization

4.3.1 Classical conditions

The synthesis of acyloxymethyl esters of LEV **14** started from optimization experiments with commercially available chloromethyl butanoate **10a**. A series of experiments were conducted with several experimental conditions including those described by *Maeda et al*[170]. Reactions were conducted by varying solvent (DMF or CH_3CN), temperature (from room temperature to 80°C), reaction time (from 17 hours to 7 days) and with or without catalyst. The results demonstrated that the best conditions were refluxing 1 equivalent LEV in the presence of 2 equivalents of K_2CO_3 with 1.2 equivalents of chloromethyl butanoate **10a** and 0.1 equivalent of TBAI in CH_3CN for 7 days (**Scheme 11**, **Table 50**).



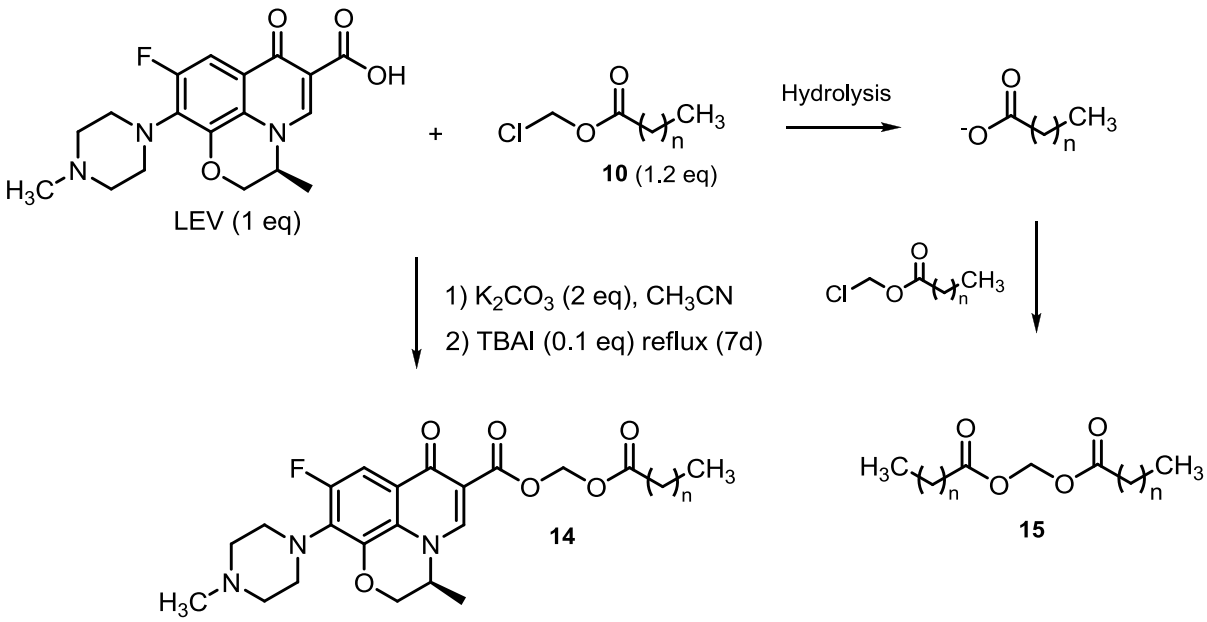
Scheme 11 Synthetic route to the target butanoyloxymethyl ester of LEV **14a**

Table 50 Optimization assays for the synthesis of butanoyloxymethyl ester of LEV (n = 2)

Base	*Solvent*	*Catalyst (relative amounts/LEV)*	*Temperature*	*Reaction time*	*Yield of **14a** (%)*
K_2CO_3[a]	DMF	–	50ºC	17h	traces
K_2CO_3[b]	DMF	–	rt	4d	23
K_2CO_3[b]	DMF	–	50ºC	7d	28
K_2CO_3[b]	CH_3CN	–	rt	4d	15
K_2CO_3[b]	CH_3CN	–	50ºC	7d	36
K_2CO_3[b]	CH_3CN	–	Reflux	7d	25
K_2CO_3[b]	**CH_3CN**	**TBAI (0.1 eq)**	**Reflux**	**7d**	**48**

[a]Experimental conditions described by *Maeda et al*[170] [b]*Sandra Dorbes, Master 2, February-June 2005*

Later on this protocol was applied to the synthesis of long-chain acyloxymethyl esters of LEV **14e-i.** However, in comparison with short-chain compound **14a**, the long-chain analogs were obtained with a significant decrease of the yield (0-10% yields) and noticeable quantities of methylene dialkanoate by-product **15** (14-35% yields) were isolated (**Scheme 12, Table 51**). The formation of these symmetric double esters may be explained by the hydrolysis of the chloromethyl alkanoates **10** leading to the formation of corresponding carboxylates which probably react more rapidly with the remaining chloromethyl alkanoate than LEV carboxylate.



Scheme 12 Synthesis of acyloxymethyl esters of LEV **14**

Table 51 Yields of long-chain acyloxymethyl esters of LEV **14e-i** and methylene dialkanoate **15e-i.**[a]

n	*Compound*	*Yield of **14** (%)*[b]	*Coumpound*	*Yield of **15** (%)*[b]
10	**14e**	5	**15e**	17
12	**14f**	10	**15f**	35
14	**14g**	3	**15g**	14
16	**14h**	5	**15h**	28
18	**14i**	0	**15i**	0

[a]*Sandra Dorbes, Master 2, February-June 2005* [b]Isolated yields after purification

In summary, classical conditions for the alkylation of LEV with chloromethyl butanoate were optimized and gave a moderate yield of 48%. However, applying these conditions for the synthesis of long-chain acyloxymethyl esters of LEV resulted in the significant drop of the yield. This was probably due to the hydrolysis of the chloromethyl alkanoates during the prolonged 7 days heating of the reaction mixture. In order to overcome these difficulties we decided to perform microwave-assisted attempts.

4.3.2 Microwave-assisted synthesis

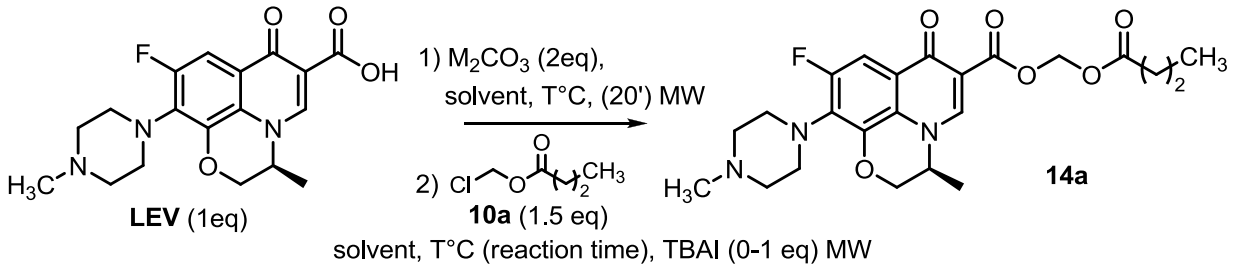
4.3.2.1 Optimization for the commercial chloromethyl butyrate

The microwave-assisted (MW) synthesis was attractive because of its major advantages such as rapid heat transfer and selective heating. This should lower the reaction time and therefore decrease the possible hydrolysis of the alkylating agent[171].

Initial attempts were carried out with chloromethyl butyrate **10a** and showed, in comparison with classical oil bath conditions, a significant decrease of the reaction time (**Scheme 13, Table 52**). In an effort to ascertain the reaction parameters governing the nucleophilic substitution, for all optimization runs (**Table 52, entries 2-13**), the reactive potassium or cesium salt was always prepared under MW in a discrete step using 20 min irradiation time from 1 equivalent of LEV and 2 equivalents of base. In the nucleophilic substitution step, 1.5 equivalents of chloromethyl butyrate was used with regard to LEV.

Reactions were first conducted in DMF at 120°C, by varying reaction time and catalyst amounts (**Table 52, entries 2-6**). Three experiments were conducted to assess the effect of the relative amounts of TBAI on the isolated yields while keeping constant the irradiation time (60 min) (**Table 52, entries 2-4**). The yield was significantly dependent on the amount of catalyst used, the use of 0.2 equivalent of TBAI led to an optimized yield of 46%. Next, the effect of irradiation time on the substitution (**Table 52, entries 4–6**) was assessed and showed that increasing the reaction time from 60 to 120 min led to a loss of yield of the expected **14a**. Esterifications with chloroalkylesters were described as more effective when the reaction was conducted in *N,N'*-dimethylpropyleneurea DMPU[172] and we have found out that it gave similar yield as in DMF (**Table 52, entry 7)**.

CH_3CN as solvent at 90°C for 60 minutes with 0.2 equivalent of TBAI, led to an enhance yield of 56% (**Table 52, entry 8)**. As observed with DMF, an increase of time of reaction from 60 to 90 minutes led to a loss of yield (**Table 52, entry 9**). We also assessed the effect of the relative amounts of TBAI. When the reaction was carried out with 0.1 equivalent of TBAI, we observed a loss of yield and with 0.3 equivalent of TBAI, the yield remained unchanged (**Table 52, entries 10-11)**. The change of the base by using Cs_2CO_3 instead of K_2CO_3, either in DMF or CH_3CN, did not lead to an improved yield (**Table 52, entries 12-13**).



Scheme 13 MW-assisted synthesis routes of butanoyloxymethyl ester of LEV **14a**

Table 52 MW vs classical conditions for the synthesis of butanoyloxymethyl ester of LEV **14a**

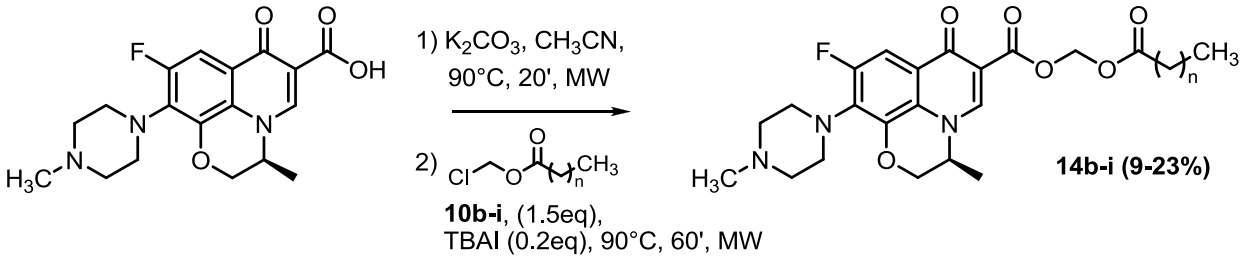
Entry	*Heating*	*Solvent*	*Base*	*Temp.*	*Time*	*Relative amount of TBAI/LEV*	*Yield (%)*
1	**Oil bath**	CH_3CN	K_2CO_3	Reflux	7d	0.1 eq	**48**
2	**MW**[a]	DMF	K_2CO_3	120°C	60'	0	8[b]
3	**MW**	DMF	K_2CO_3	120°C	60'	1	7[b]
4	**MW**	**DMF**	**K_2CO_3**	**120°C**	**60'**	**0.2**	**46**[b]
5	**MW**	DMF	K_2CO_3	120°C	90'	0.2	47[b]
6	**MW**	DMF	K_2CO_3	120°C	120'	0.2	20[b]
7	**MW**	DMPU	K_2CO_3	120°C	120'	0.2	16[b]
8	**MW**	**CH_3CN**	**K_2CO_3**	**90°C**	**60'**	**0.2**	**56**
9	**MW**	CH_3CN	K_2CO_3	90°C	90'	0.2	32
10	**MW**	CH_3CN	K_2CO_3	90°C	60'	0.1	46
11	**MW**	CH_3CN	K_2CO_3	90°C	60'	0.3	55
12	**MW**	DMF	Cs_2CO_3	120°C	60'	0.2	37
13	**MW**	CH_3CN	Cs_2CO_3	90°C	60'	0.2	45

[a]All reactions were performed in 80 mL cylindrical Pyrex open vessels [b]Previous attempts

Then, the optimal MW conditions for the synthesis of butanoyloxymethyl ester **14a** were found to be the use of 0.2 equivalent of catalyst (TBAI) in CH_3CN at 90°C for 60 minutes in the presence of K_2CO_3.

4.3.2.2 Synthesis of a series of acyloxymethyl esters of LEV

We then applied this experimental protocol to the synthesis of the series of acyloxymethyl esters LEV **14b-i** with longer chain lengths. Potassium salt of LEV was prepared by reaction of K_2CO_3 (2 equivalent) on LEV (1 equivalent) in CH_3CN at 90°C under MW using 20 min irradiation time. After the addition of TBAI catalyst (0.2 eq) and chloromethyl alkanoates **10b-i** (1.5 eq), the mixture was irradiated one hour more at 90°C. Though yields were somewhat higher (from 10 to 23% yields) for the synthesis of **14e-i** than those obtained under oil bath heating (from 3 to 10% yields), they were significantly lower than the yields obtained for the short chain analog **14a** (n=2). (**Scheme 14**, **Table 53**)



Scheme 14 MW-assisted synthesis of acyloxymethyl esters of LEV **14b-i**

Table 53 Yields of acyloxymethyl esters of LEV **14b-i** under MW conditions

Compound	*n*	*Yield, %*
14b	4	16
14c	6	18
14d	8	16
14e	10	17
14f	12	9
14g	14	11
14h	16	23
14i	18	–[a]

[a]Failed to isolate the pure product

In order to investigate the reason for these poor yields, we decided to examine the byproduct formation. A representative example of long-chain derivatives was chosen for this

study, that was the MW-assisted synthesis of octadecanoyloxymethyl ester of LEV **14h.** Indeed, during the synthesis of acyloxymethyl esters of LEV conducted under classical oil-bath conditions, high quantities of methylene dialkanoates by-product **15** were observed and were thought to be the possible cause of the low yield (**Table 51**).

In order to clarify the reaction behavior we have evaluated the rate of by-products, that were methylene dioctadecanoate **15h**, octadecanoic acid (arising from the hydrolysis of chloromethyl octadecanoate) along with unreacted reagent **10h** and the desired octadecanoyloxymethyl ester of LEV **14h** (**Table 54**). After a tedious purification, we have got many batches containing pure product or a mixture of two products. In this last case, the yields have been estimated by ^{1}H NMR spectroscopy. We observed that under MW conditions, much less of the symmetric methylene dioctadecanoate **15h** and more of the desired octadecanoyloxymethyl ester of LEV **14h** were produced in a shorter time of reaction comparing to classical oil-bath conditions. However, we have seen that the main part of the alkylating agent **10h** remains unreacted or hydrolyzed to octadecanoic acid. In addition, unreacted LEV was also detected.

Table 54 Conversion of chloromethyloctadecanoate **10h** during the synthesis of long-chain compound (n = 16)

Compound	*Conversion[a], %*	
	Microwave, 1 hour	*Oil-bath, 7 days*
14h	18[b]	3[b]
n-$C_{17}H_{35}CO_2CH_2Cl$, **10h**	32[c]	–[d]
(n-$C_{17}H_{35}COO)_2CH_2$, **15h**	5[c]	28[b]
n-$C_{17}H_{35}CO_2H$	45[e]	–[d]

[a]Based on the *n*-$C_{17}H_{35}CO_2CH_2Cl$ (Its initial quantity is set to 100%) [b]Isolated product [c]Estimated by ^{1}H NMR spectroscopy for the mixture of **10h** and **15h** [d]Was not evaluated during experiment [e]Calculated as a difference between the initial quantity of *n*-$C_{17}H_{35}CO_2CH_2Cl$ and other C17 containing components

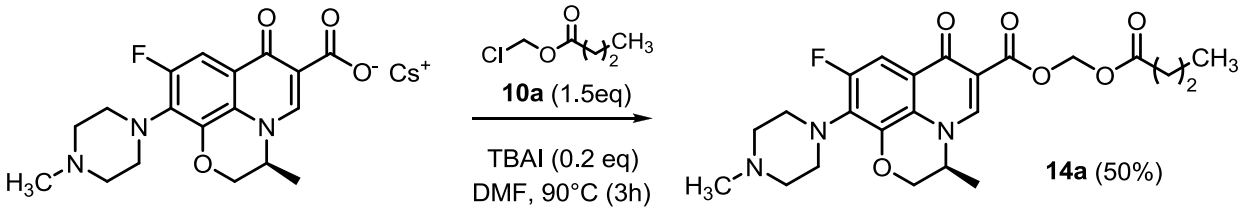
In summary, the MW-assisted synthesis of acyloxymethyl esters of LEV had some advantages over classical conditions (significantly lower reaction time) but the yields were low for the long-chain derivatives. This could be explained by the relatively high hydrolysis rate of chloromethylalkanoate. The presence of unreacted LEV and alkylating agent may be due to their

low reactivity in these conditions, which is supposed to be the second reason for the low yield. So we decided to search for alternative conditions in order to optimize this synthetic protocol.

4.3.3 Modified classical oil-bath conditions: use of cesium salts of LEV

4.3.3.1 Preliminary attempt for butyloxymethyl ester of LEV (n = 2)

Cesium salts generally display higher solubility in organic solvents. We first isolated LEV cesium salt after refluxing LEV with cesium carbonate in dichloromethane. DMF was first evaluated as solvent. The other parameters were kept the same as for the microwave and classical conditions: 90 ºC, 0.2 equivalent of TBAI and 1.5 equivalents of chloromethyl butyrate **10a** (**Scheme 15**). Initial experiment rendered the desired butyloxymethyl ester of LEV **14a** in moderate yield (50%) after only 3 hours while classical conditions discussed above required 7 days under reflux to achieve 48% yield.



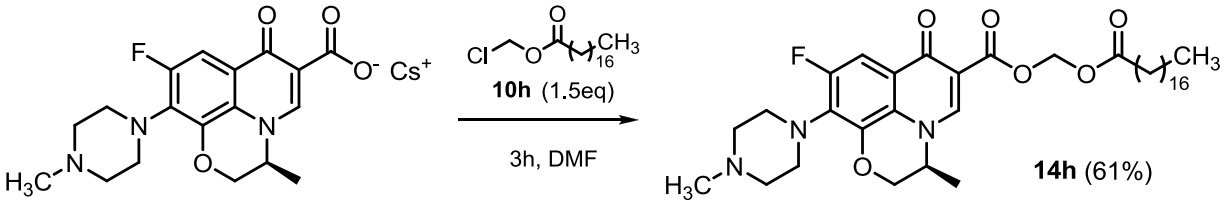
Scheme 15 Synthesis of butyloxymethyl ester of LEV in DMF by using cesium salt

This reaction conducted with cesium salt of LEV seemed to be faster than the one from LEV. However, it should be pointed out that this procedure, when applied to a series of long chain derivatives (bearing 11-19 carbon atoms), was always plagued with difficulties during purification. Generally, several (2–3) silica gel columns (with different solvent systems) were necessary to isolate the lipophilic esters in pure form without any trace of TBAI. Low isolated yields were thus obtained. To circumvent this problem, the reaction with chloromethyl octadecanoate was further investigated using oil-bath heating and without TBAI.

*4.3.3.2 Preliminary attempts for the synthesis of octadecanoyloxymethyl ester of levofloxacin **14h** (n = 16)*

We decided to optimize the reaction on a long-chain derivative, as the increase of chain length of alkylating agents posed greater problems. The reaction was conducted with

chloromethyloctadecanoate **10h** in the same conditions, except we did not use any catalyst (**Scheme 16**). In order to evaluate the optimal reaction time, the main product formation was monitored by HPLC. Thus, during the first hour, probes were taken each 20 minutes and the concentration of cesium salt of LEV was determined at the same time as that of the formed compound **14h** (**Table 55**). Conversion of cesium salt of LEV to the main product **14h** was evaluated to be around 64% after 60 minutes. The mixture was then allowed to react for 2 more hours. The isolated yield after this total time of 3 hours was 60% which is significantly higher than ever before but was appreciably the same to the evaluated yield after 1 hour of reaction.



Scheme 16 Synthesis of octadecanoyloxymethyl ester of LEV **14h**

Table 55 Reaction monitoring for the synthesis of **14h**

	Concentration (μM) determined by HPLC	
Time, min	***14h***	*Cesium salt of LEV*
0[a]	0	0.440
20	0.257	0.186
40	0.263	0.183
60	0.263	0.180

[a]Calculated values before the start of the reaction

During the purification of **14h**, once more, we have isolated methylene dioctadecanoate **15h** and octadecanoic acid along with unreacted alkylating agent **10h**. As shown in **Table 56**, we observed that, in comparison with the previously discussed MW-assited synthesis (**Table 54**), the obtained higher yield could be explained by a two-fold higher conversion of the chloromethylalkanoate **10h** into the main product **14h**. Though we could still observe hydrolysis products, the current conditions had positive impact on the yield.

Table 56 Comparison of conversion of chloromethyloctadecanoate **10h** for the current conditions and MW-assisted synthesis of **14h** (n = 16)

Compound	*Conversion[a], %*	
	Current conditions, 3 hours	*MW, 1 hour*
14h	34[b]	18[b]
n-$C_{17}H_{35}CO_2CH_2Cl$, **10h**	17[c]	32[c]
$(n\text{-}C_{17}H_{35}COO)_2CH_2$, **15h**	4[c]	5[c]
n-$C_{17}H_{35}CO_2H$	45[d]	45[d]

[a]Based on the *n*-$C_{17}H_{35}CO_2CH_2Cl$ (Its initial quantity is set to 100%) [b]Isolated product [c]Estimated by ^1H NMR spectroscopy for the mixture of **10h** and **15h** [d]Calculated as a difference between the initial quantity of *n*-$C_{17}H_{35}CO_2CH_2Cl$ (1.5 eq) and other C17 containing components

A series of small-scale experiments were conducted in order to find the best conditions of reaction. The yields of compound **14h** were estimated by HPLC analysis of the crude reaction mixture. Thus, we assessed the effect of CH_3CN instead of DMF (**Table 57**, **entries 1, 2**), the time of reaction (**Table 57**, **entries 1, 3**) and the amount of chloromethyloctadecanoate (**Table 57**, **entries 1, 4**).

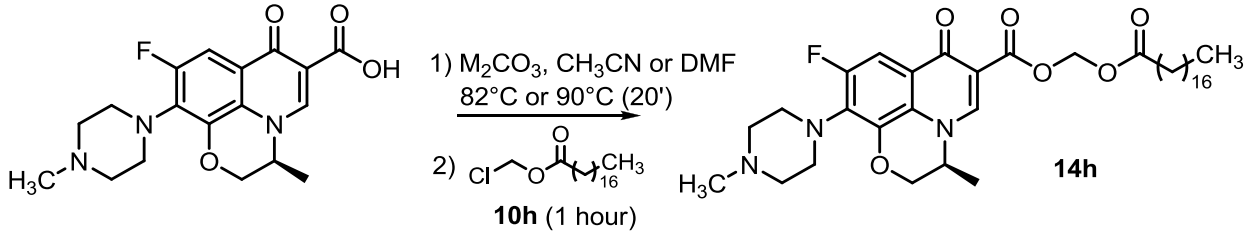
Table 57 Optimization of reaction conditions for the synthesis of **14h**

Entry	*Time (hour)*	*Solvent*	*RCO_2CH_2Cl (eq)*	*Yield (%)*
1	3	DMF	1.5	64
2	3	CH_3CN	1.5	58
3	1	DMF	1.5	70
4	1	DMF	1	63

First of all, we observed that CH_3CN gave lower yield than DMF. As already observed the reaction time in DMF can be decreased to 1 hour. Using 1 equivalent of the alkylating agent instead of 1.5 equivalents slightly lowered the yield, but facilitated the purification step. Though an increased yield was achieved, this protocol still presented some drawbacks: the step of the cesium salt preparation and the moisture sensitivity of the cesium salt making it uneasy to handle. The resolution of these issues will be discussed in the next section.

4.3.4 Modified classical conditions: in situ formation of LEV salts

In order to overcome these drawbacks, we decided to prepare *in situ* the cesium (or potassium) salt of LEV. During an optimization study, isolated yields of compound **14h** were determined. A mixture of LEV and M_2CO_3 (1 equivalent) was heated in CH_3CN or DMF for 20 minutes. After adding octadecanoyloxymethyl chloride, the reaction was performed during 1 hour (**Scheme 17**). The results obtained for various parameters: the solvent (DMF or CH_3CN), the base (Cs_2CO_3 or K_2CO_3) and the quantity of alkylating agent (1 or 1.5 equivalents) are presented in **Table 58.**



Scheme 17 Synthesis of octadecanoyloxymethyl ester of LEV **14h**

Table 58 Optimization runs for the synthesis of **14h**

Base, eq	*Solvent*	*RCO_2CH_2Cl, eq*	*Yield, %*
Cs_2CO_3 (1eq)	DMF	1	83
Cs_2CO_3 (1 eq)	CH_3CN	1	23
K_2CO_3 (1 eq)	**DMF**	**1**	**81**
K_2CO_3 (1 eq)	CH_3CN	1	9
K_2CO_3 (1 eq)	DMF	1.5	86

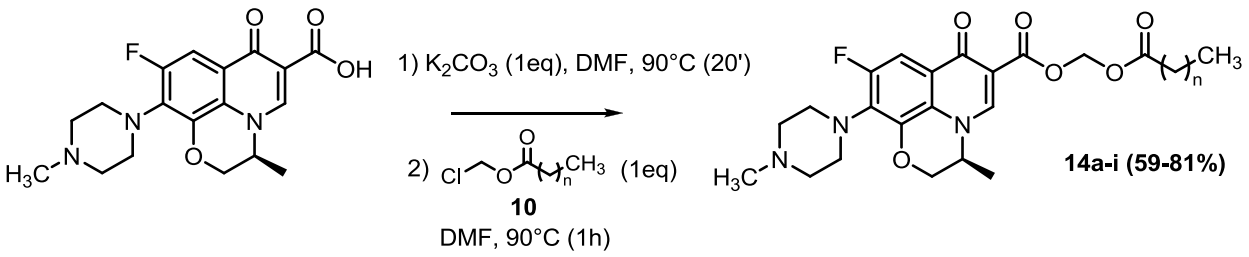
From obtained results, we observed that the use of *in situ* salts formation increased the yield and favored the conversion of LEV into the desired compound **14h**. The nature of the base used was not really important in this case as both Cs_2CO_3 and K_2CO_3 gave similar yields, if the reaction was conducted in DMF. The nature of the used solvent turned out to be the determining factor for this reaction: when DMF was used, the isolated yields were four- to eight-folds higher than those in CH_3CN. This phenomenon could be explained by the preferred cation-solvation by DMF, thereby activating the LEV carboxylate-anion. A poor difference was obtained between equimolar and 1.5 equivalent quantities of the chloromethyloctadecanoate. We selected as optimal conditions for the synthesis of the rest of acyloxymethyl esters of LEV derivatives: 1

equivalent of K_2CO_3 (cheaper and less hygroscopic), DMF as solvent, 1 equivalent of alkylating agent RCO_2CH_2Cl, 1 hour reaction time at 90°C.

4.3.5 Synthesis of acyloxymethyl esters of LEV derivatives using optimized conditions

We have synthesized the series of LEV acyloxymethyl esters **14a-i** in yields varying from 59 to 81% depending on the length of the chain (**Table 59**). ^{1}H NMR spectra of the crude mixture showed no significant difference between long and short chain derivatives. As the lower yields were obtained for compounds with shorter chains, we proposed that as they are more polar they are probably held tighter by the silica column during the purification step.

Table 59 Yields of acyloxymethyl esters of LEV



Compound	*n*	*Yield, %*
14a	2	59
14b	4	69
14c	6	75
14d	8	79
14e	10	79
14f	12	77
14g	14	79
14h	16	81
14i	18	79

In summary, we have designed and synthesized the series of acyloxymethyl esters of LEV. We have tried three general synthetic protocols while optimizing the experimental conditions. During these experiments we have deduced the most important factors influencing the reaction: the reaction time (1h) and the nature of the solvent (DMF). The other factors such as the nature of the base (K_2CO_3 vs Cs_2CO_3) and the quantity of the alkylating agent (1 equivalent versus 1.5 equivalents) showed minor influence on the yield. As it was previously mentioned this compounds could represent prodrugs of LEV. Though acyloxymethyl esters of

LEV are more stable than simple esters, they are sensible to hydrolysis. So we have shifted our attention to the synthesis of "mono-" and "di-amide" derivatives of LEV with higher stability towards enzymatic and chemical hydrolysis.

4.4 Synthesis of C-6-(alkylcarboxamide)-linked LEV "monomers" and "dimers"

4.4.1 Synthesis of "monomer" dodecylcarboxamide derivative of LEV

As previously outlined for the synthesis of CIP derivatives, FQs did not tolerate the use of thionyl chloride or oxalyl chloride due to the high degree of degradation of FQs with these chlorinating agents.

Carbodiimides were previously used for the synthesis of CIP derivatives. In the previous section 3.2 we discussed some methods for formation of the amide bond in peptide chemistry. Carbodiimides are often used for the coupling of aminoacids and amide formation from aliphatic acids.

We compared two methods of carboxylic acid activation : i) the use of the salt of uronium with a non-nucleophilic anion which exists in the *O*- and *N*-forms: 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5-b]pyridinium hexafluorophosphate 3-oxide (*N*-HATU) and N-[(1H-1,2,3-triazolo[4,5-b]pyridin-1-yloxy)(dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate (*O*-HATU)[173] and ii) the use of the triphenylphosphine (TPP) / trichloroacetonitrile[174] (TCA) system.

It was previously reported that HATU is very efficient in solution synthesis[175] and tertiary amines such as diisopropylethylamine (DIPEA) have been considered as practically useful bases due to their non-nucleophilic property, while the highly hindered 2,4,6-trimethylpyridine (TMP) was recommended by *Carpino*[176]. Both *O*- and *N*-forms serve as excellent coupling agents. (**Figure 39**).

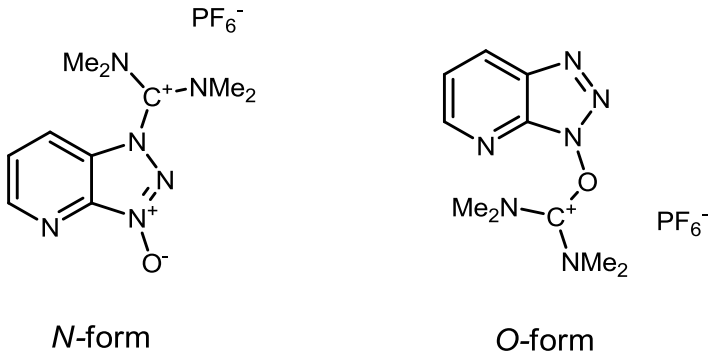


Figure 39 Two isomers of HATU coupling agent

The HATU based amide coupling is mediated in two steps, activation and coupling. Mechanistically, it is similar to the previously discussed EDCI/HOBt system with the difference that the carbodiimide moiety and nucleophilic additive are present in the same molecule of HATU (**Figure 40**). During the activation step that takes place in a basic media, HATU reacts with the carboxylate, forming an uronium intermediate**,** which is subsequently transformed to *O*- and *N*-acyl species. The more active *O*-form is generated more rapidly and is subsequently isomerized to the less active *N*-form. During the coupling step created intermediates react with the amino component to form the amide bond[177].

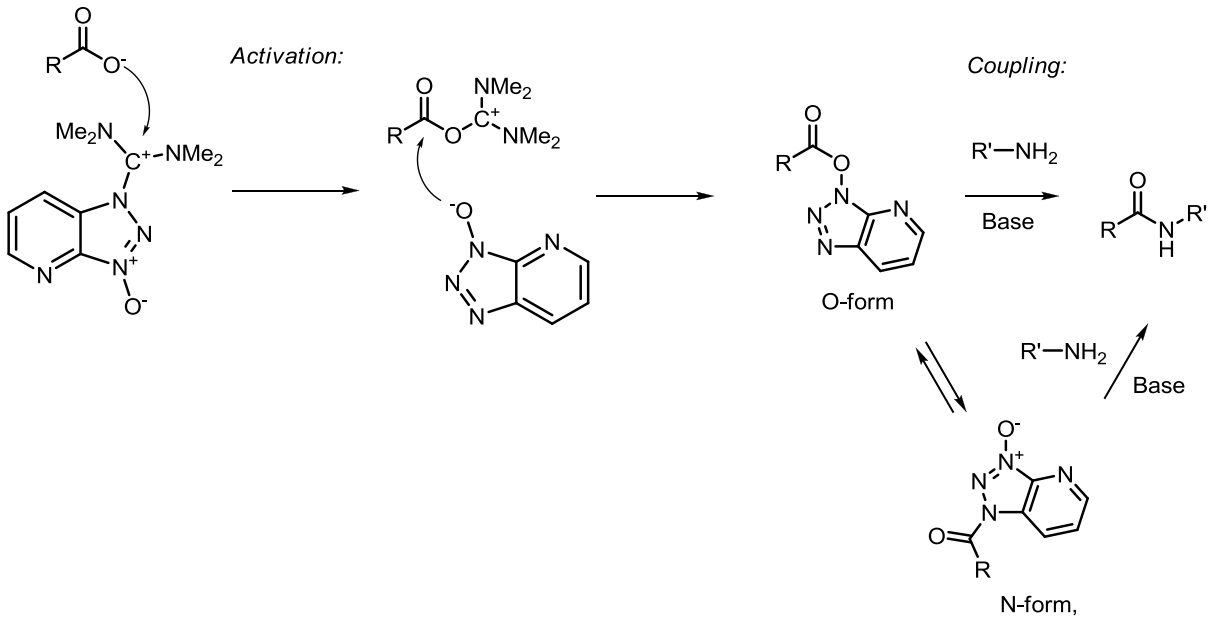


Figure 40 Mode of formation of amide bond by using HATU

TCA is an inexpensive, stable and commercially available compound, which has been used as a source of chlorine in synthetic transformations[174, 178]. The reaction of TCA with TPP readily generates triarylphosphonium chloride which can react with carboxylic acids to produce triphenylacyloxyphosphonium species. The subsequent attack of chloride anion yields acid chloride and TPP oxide[178]. This provides a mild and efficient procedure for the conversion of carboxylic acids into acid chlorides under neutral conditions (**Figure 41**)[174, 179]. Obtained acyl chloride can used to generate the desired amide by *in situ* condensation with alkylamines in the basic media.

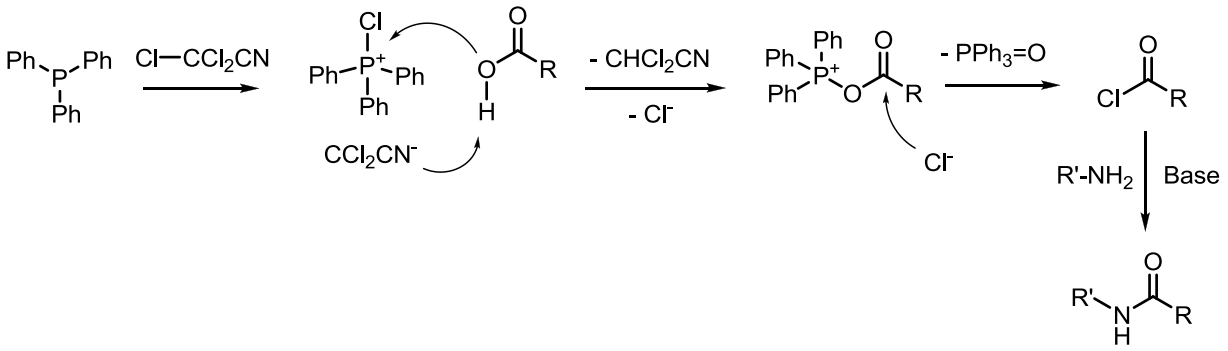
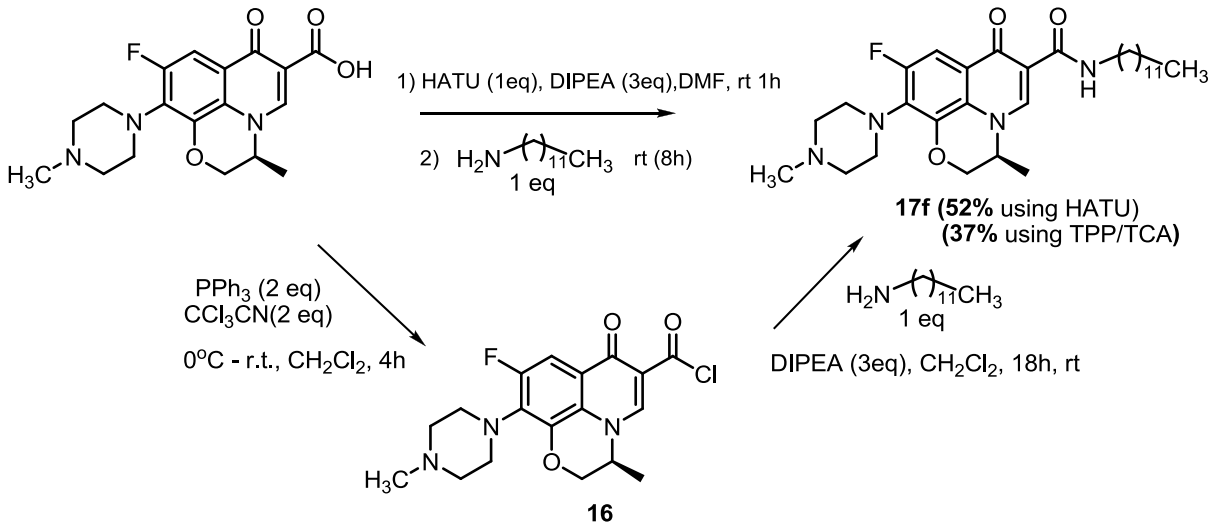


Figure 41 Mechanism of formation of amide bond TPP/TCA system

We applied these two methods to the synthesis of dodecylcarboxamide derivative of LEV **17f** (**Scheme 18**).



Scheme 18 Synthesis of dodecylcarboxamide derivative of LEV **17f**

The initial activation of the LEV by 1 equivalent of HATU and 3 equivalents of DIPEA in DMF for 1 hour at room temperature was followed by addition of 1 equivalent of dodecylamine. After stirring reaction mixture at room temperature for 8 hours, the target compound **17f** was isolated with 52% yield. Reaction went smoothly and the purification didn't pose any problems.

Acyl chloride of LEV **16** was prepared after the treatment of LEV with TPP/TCA (2 equivalents / 2 equivalents) in CH_2Cl_2 for 4 hours at room temperature. This derivative was filtered off and used in the next step without purification. Reaction with 1 equivalent of dodecylamine in the presence of 3 equivalents of triethylamine for 18 hours in CH_2Cl_2 at room

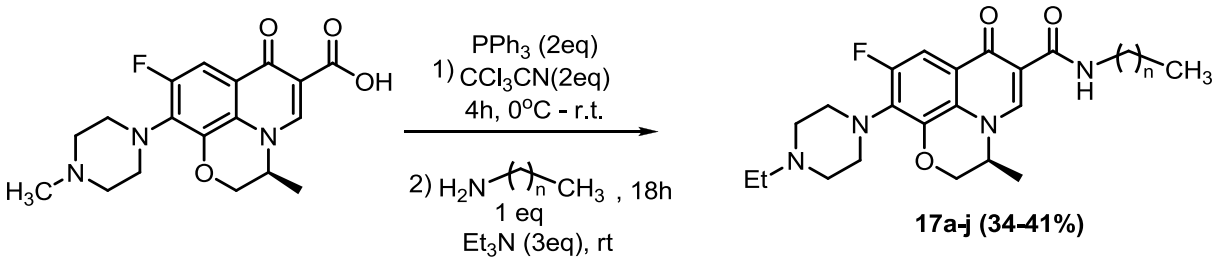
temperature provided the desired dodecylcarboxamide derivative of LEV **17f** with moderate overall yield (38%) after an easy purification (**Scheme 18**).

TPP/TCA system was chosen for the synthesis of C-6-(alkylcarboxamide)-linked LEV "monomers" and "dimers" which will be described in the next section.

4.4.1.1 Synthesis of "monomer" alkylcarboxamide derivatives of LEV

The series of "monomer" alkylcarboxamide derivatives of LEV **17a-j** was obtained, by using TPP/TCA system, with moderate yields. The summary of these results is presented in **Table 60**.

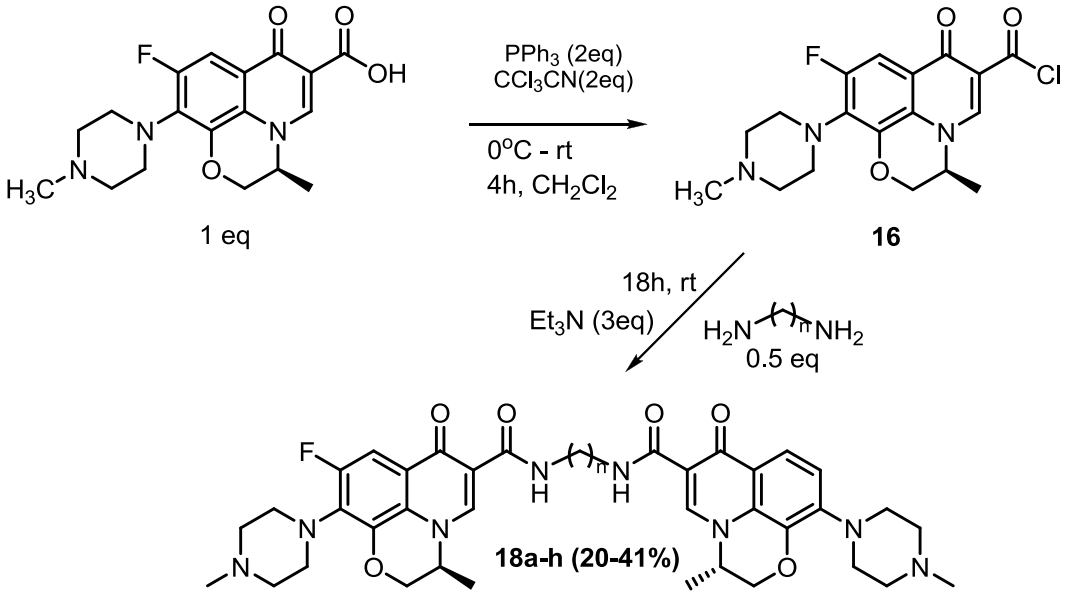
Table 60 Synthesis of "monomer" alkylcarboxamide derivatives of LEV **17a-j**



Compound	*n*	*Yield, %*
17a	3	34
17b	5	37
17c	6	36
17d	7	36
17e	9	35
17f	11	37
17g	12	39
17h	13	39
17i	14	40
17j	16	41

4.4.1.2 Synthesis of "dimer" alkylcarboxamide derivatives of LEV

For the synthesis of "di-amide" derivatives of LEV **18**, the same TPP/TCA system was used. The "dimeric" derivatives were obtained with yields somewhat lower (20-41%) than that for their "monomeric" analogs **17** (**Scheme 19, Table 61**).



Scheme 19 Synthesis of "dimer" alkylcarboxamide derivatives of LEV **18a-h**

Table 61 Yields of "dimer" alkylcarboxamide derivatives of LEV **18a-h**

Compound	*n*	*Yield*
18a	4	39
18b	5	29
18c	6	41
18d	7	37
18e	8	23
18f	9	20
18g	10	27
18h	12	27

In summary, during this work, five series of LEV and CIP derivatives were prepared (**7**, **9**, **14**, **17**, **18**). Several synthetic challenges were faced on the way to these new compounds and a number of optimization studies were conducted in order to overcome these problems.

5. Stability tests

The stability of the biologically active compounds is a very important physicochemical property. It determines the rate of degradation of the products and therefore influences the pharmacokinetics: maximum concentration in the serum and AUC (area under the curve concentration/time). Therefore stability evaluation step is inevitable during the drug development process. Different types of stability tests are necessary for "hits" – newly synthesized compounds

with biological activity. The simplest initial tests measure the stability *in vitro* - in the water buffer at physiological pH = 7.4 and 37ºC. These tests could provide some insight into the stability of the compounds and select the successful candidates for *in vivo* testing.

Though there is no standard universal procedure for stability evaluation of every compound, analytical HPLC is one the most frequently used methods[180]. It is relatively simple and quick with a high potential of separating complex mixtures of compounds. The optical ultraviolet (UV) detection provides quantification and a high sensitivity of this method.

So we decided to use the HPLC procedure and UV detection to measure the stability of our compounds.

5.1 Conditions for the stability testing

Though there are a number of examples of experimental conditions for the stability testing[180] there is no generalized procedure recognized as a standard. We have chosen phosphate buffer saline (PBS) as a buffer solution for the testing. Our compounds weren't soluble directly in the buffer so mixture of PBS/acetonitrile (50:50) was used. Initially compounds were dissolved in the DMSO and then they were diluted by the PBS/acetonitrile mixture. Experimental evidences from the literature showed that up to 10% of DMSO doesn't result in significant changes of stability[180a]. However, even in these conditions the most liphophilic compounds weren't soluble. We decided to test synthesized compounds for 6-7 days with intermediate sampling.

5.2 Results

5.2.1 CIP derivatives

We have selected representatives from each series with wide range of chain lengths. In a typical experiment 1mM DMSO solution of compound was diluted by PBS to render the final concentration 10^{-4}M of tested compound and 10% of DMSO. An aliquot was taken and kept at 4ºC (0 day). Then the vial was sealed and incubated for 6 days at 37ºC (6 days). In order to evaluate the degradation degree of tested compounds these two probes were injected. Chromatograms were extracted at the wavelength λ = 280 nm as it represents an absorbance maximum for these series of CIP derivatives. By the comparison of the initial chromatogram and the one after the incubation we could estimate the percentage of the remaining parent compound relative to the initial concentration. Typical chromatograms for 0 day and 6 days probes are demonstrated on the **Figure 42**. The peak with t_r = 1.0 min is associated with the dead volume of the column. The series of "monomeric" CIP derivatives can provide two possible degradation

products: CIP (retention time t_r = 1.6 min) and **4** (t_r = 2.6 min). The presence of **4** is expected for the series of "mono-oxoethylesters" CIP derivatives **3**.

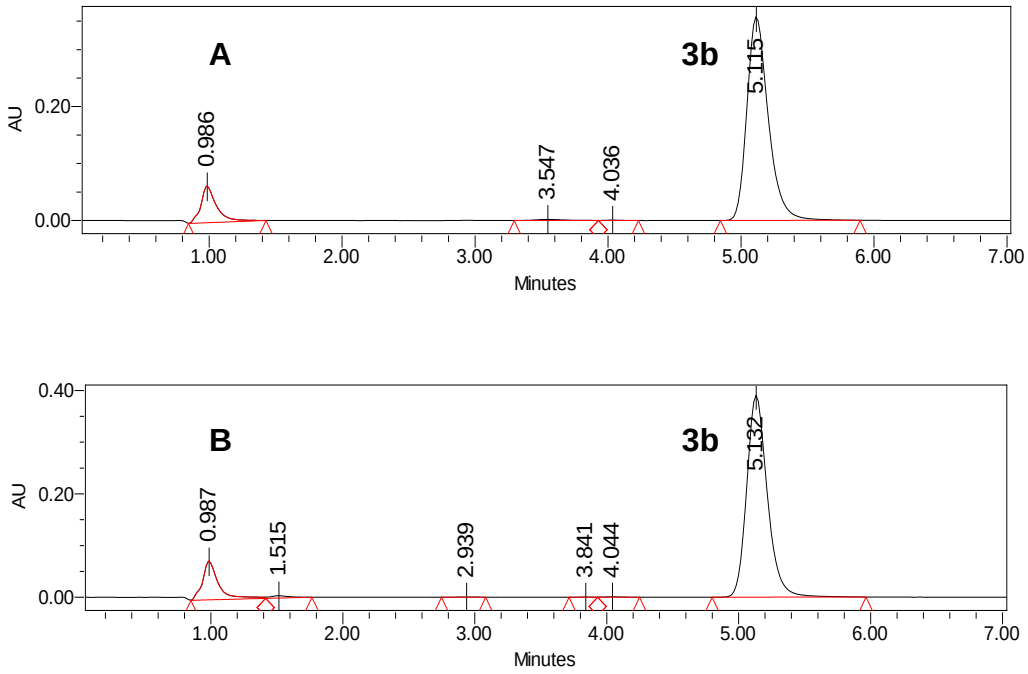
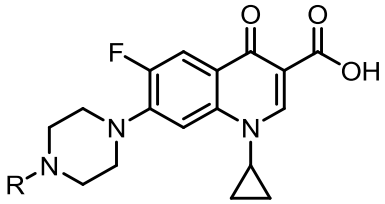


Figure 42 Representative example of 0 day (A) and 6 days (B) probes for **3b**

As we can see from the results the CIP "monomeric" derivatives (**1**, **2**, **3** and **6**) are stable at described conditions with more than 97% of the unhydrolyzed compound after 6 days of incubation (**Table 62)**.

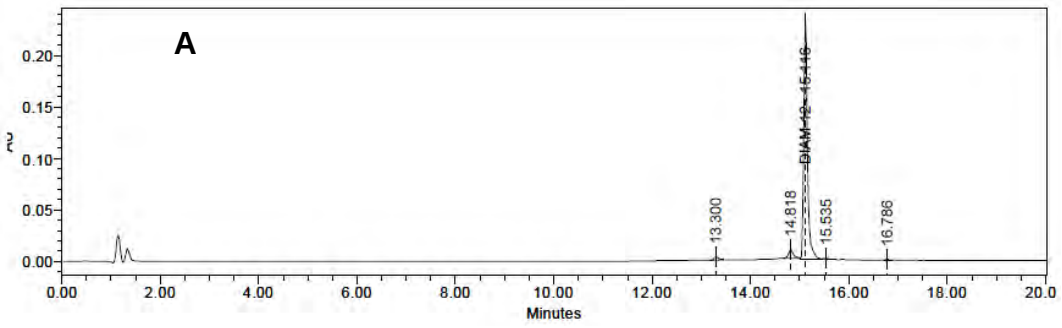
Table 62 Stability of CIP "monomeric" derivatives



Compound	*R*	*Stability[a], %*
		6 days
1f	$-CO(CH_2)_5CH_3$	100
1h	$-CO(CH_2)_8CH_3$	100
1k	$-CO(CH_2)_{14}CH_3$	100
2	$-COCH_2Cl$	>99
3b	$-COCH_2OCO(CH_2)_2CH_3$	100
3c	$-COCH_2OCO(CH_2)_4CH_3$	>99
3d	$-COCH_2OCO(CH_2)_6CH_3$	>97
6a	$-C(O)OCH_2CH_3$	100
6b	$-C(O)O(CH_2)_3CH_3$	100
6c	$-C(O)O(CH_2)_7CH_3$	100
6g	$-C(O)OC(CH_3)_3$	>99

[a]Stability(%) at x day = [mean measured concentration (n = 2) at x day/mean measured concentration (n = 2) at 0 day] × 100

During stability studies of "dimeric" CIP derivatives we have monitored the intermediate concentration at 3 and 5 days point in order to estimate the rate of hydrolysis. We have also evaluated the presence of CIP in the solution during stability testing. However, for all compounds the peak corresponding to CIP was not observed (**Figure 43**) and the data shown on the **Table 63** proves that CIP "di-amide" derivatives **7** exhibit no hydrolysis after 7 days of incubation.



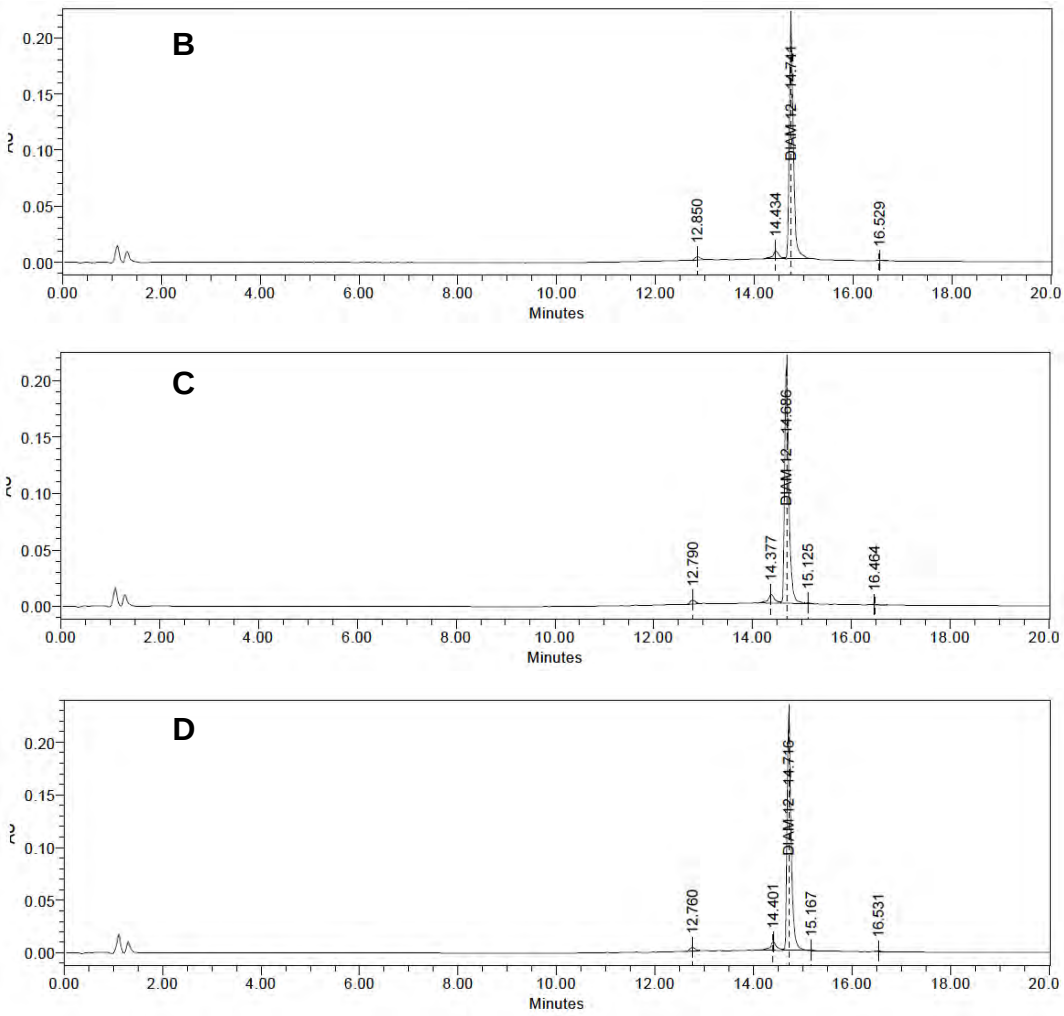
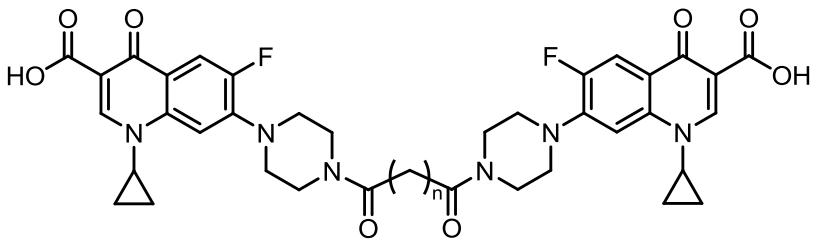


Figure 43 Representative examples of chromatograms for 0 day (A), 3 days (B), 5 days (C) and 7 days (D) probe for **7c** (n = 12).

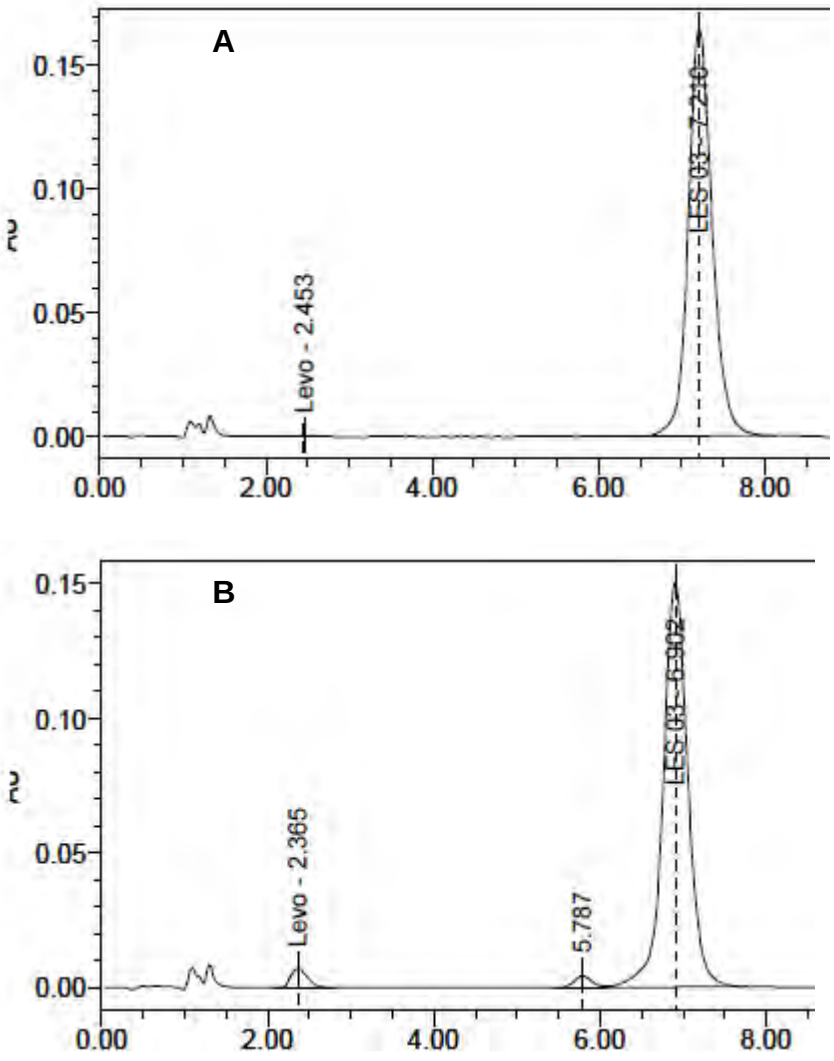
Table 63 Stability of CIP "di-amide" derivatives



Compound	*Stability, %*		
	3 days	*5 days*	*7 days*
7b (n = 10)	100	100	100
7c (n = 12)	100	100	100
7e (n = 14)	99	>99	>99

5.2.2 LEV derivatives

For the stability testing of LEV derivatives we have utilized similar conditions as for CIP derivatives: PBS/acetonitrile buffer (pH = 7.4), 10% DMSO, 7 days incubation at 37ºC. After the incubation we have noticed the presence of other peaks which didn't correspond to tested compounds, nor to the hydrolysis product. Having investigated their UV-spectra it was proposed that they are plasticizers from the rubber septum, like *o*-dialkylphthalates. Also due to the overlapping with tested compounds we have to change the detection wavelength to 325 nm for **14g**. Because of the low solubility of the most lipophilic compounds in the series we have to increase dilution to $5 \cdot 10^{-5}$M for **14e** and $1 \cdot 10^{-5}$M for **14g**. We have also monitored the LEV (retention time t_r = 2.4 min) concentration during the incubation. As we can see from these results the LEV "mono-oxoethylesters" series **14** are only slightly hydrolyzed in current conditions with more than 92% of the final compound after 7 days of incubation (**Figure 44, Table 64**).



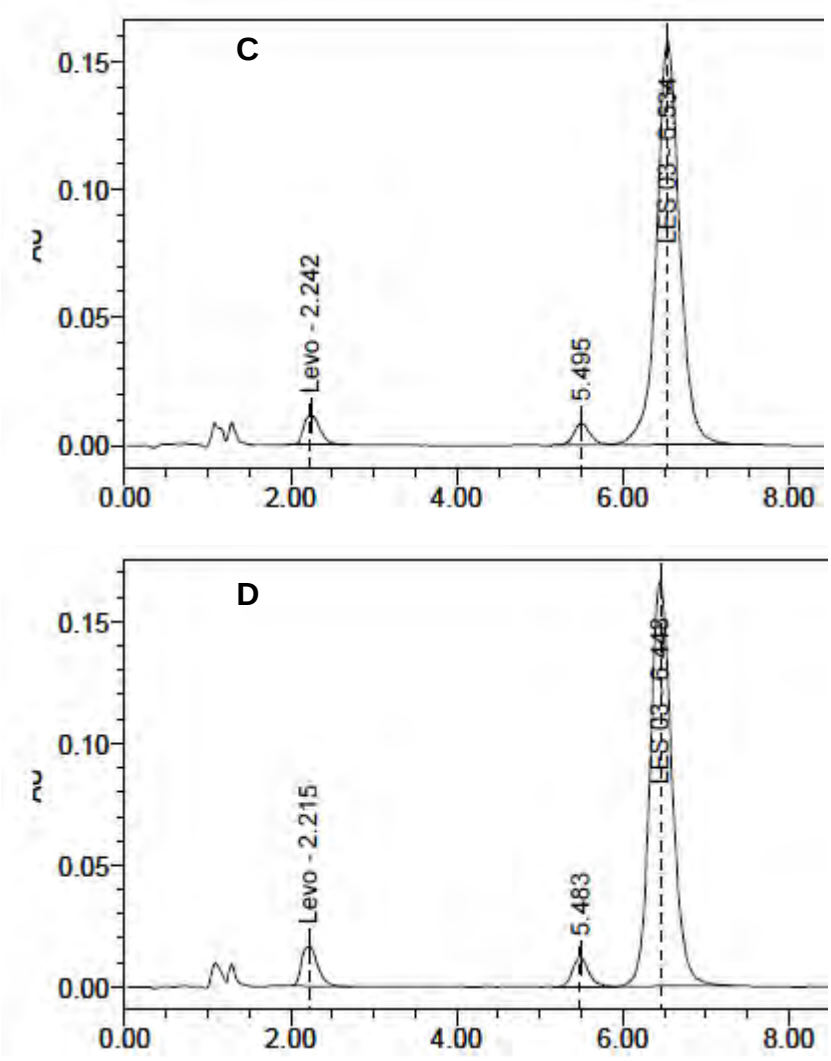
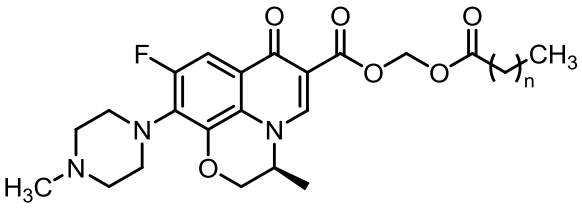


Figure 44 Representative examples of chromatograms for 0 day (A), 3 days (B), 5 days (C) and 7 days (D) probe for **14a** (n = 2)

Table 64 Stability of LEV acyloxymethyl esters



Compound	*Stability, %*			
	0 days	*3 days*	*5 days*	*7 days*
14a (n = 2)	100	95	93	92
14c (n = 6)	100	98	94	92
14e (n = 10)	100	96	93	93
14g (n = 14)	-	95[a]	93[a]	93.0[a]

[a]The **14g** peak was slightly overlapped with impurity peak so we had to change the wavelength of the UV detector.

For the LEV "mono-amide" series **17** we have used glass stoppers and didn't observe the other peaks. The results showed minor LEV quantities or no LEV in the mixture after 7 days, so we can conclude that compounds from LEV "mono-amide" series are also stable at these conditions (**Table 65**, **Figure 45**).

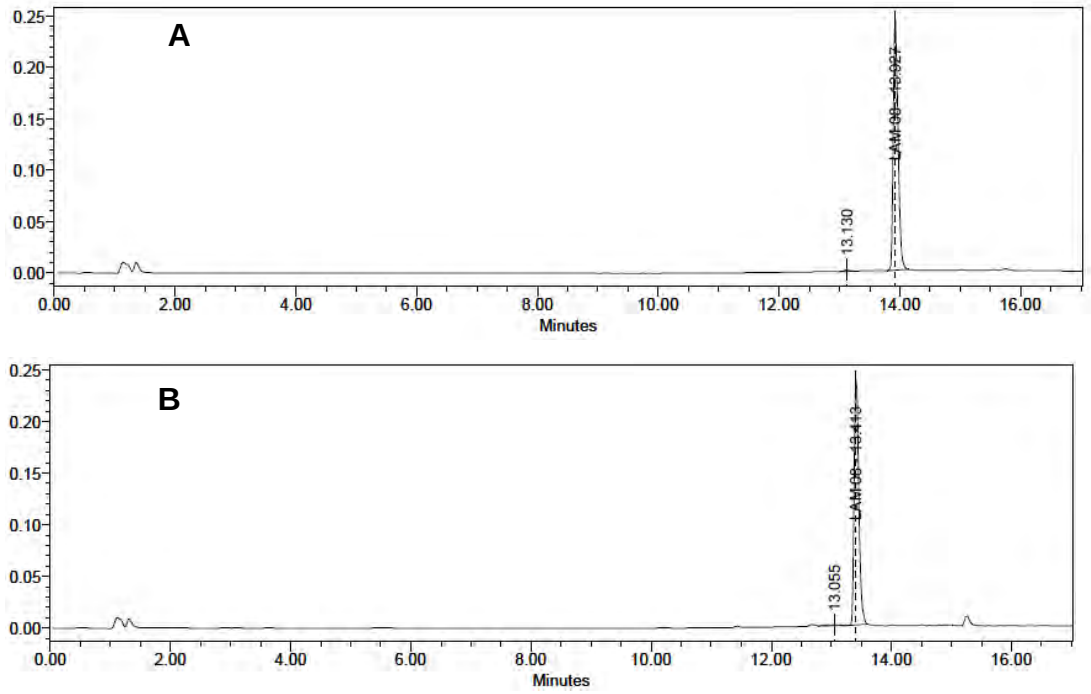
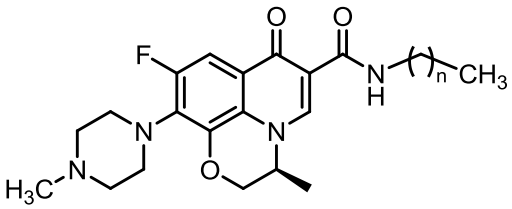


Figure 45 Representative examples of chromatograms for 0 day (A) and 7 days (B) probe for **17d** (n = 7)

Table 65 Stability of LEV "mono-amide" derivatives



Compound	*Stability, %*	
	0 days	*7 days*
17a (n = 3)	98	95
17b (n = 5)	100	100
17d (n = 7)	99	99
17h (n = 13)	100	100

In summary, all tested compounds were found to be quite stable at pH = 7.4 and 37ºC with higher than 92% of unhydrolyzed compound remained after incubation. The least stable compounds were LEV acyloxymethyl derivatives which showed noticeable amounts of the hydrolysis product. This could be explained by the fact that they possess acyloxymethyl ester bound which is more easily hydrolyzed than the amide bond. Indeed, the other series of compounds containing amide bond didn't exhibit significant hydrolysis. After stability evaluation of synthesized compound we have switched on the biological testing which will be described in the next chapter.

6. Conclusion

In summary, during the thesis we have prepared five series of LEV and CIP derivatives (**7**, **9**, **14**, **17**, **18**). During the synthesis of 7-(4-(oxoethylalkanoate)piperazin-1-yl)-linked CIP "dimers" **9** we have faced the problem of the low reactivity of N-chloroacetylciprofloxacin so we conducted optimization studies with varying solvent, temperature, catalyst quantity and base. We have found that Cs_2CO_3 in the DMF with equimolar quantities of TBAI provide the best combination in the terms of reaction time and yield. The synthesis of 7-(4-(alkanoyl)piperazin-1-yl))-linked CIP "dimers" **7** required the implication of EDCI coupling agent with HOBt additive to achieve high yields and facilitate the purification.

The C-6 LEV modification was also complicated by the low reactivity of its carboxyl moiety. For the synthesis of C-6-acyloxymethyl esters of LEV **14** we have tried microwave conditions to decrease the reaction time in comparison with original thermal pathway. However, we have found that after several optimizations (Cs_2CO_3, DMF) thermal conditions were capable to render the desired compounds within 1hour and with good yields (>59%). We have also faced several synthetic challenges on the way to C-6-alkylcarboxamides of LEV **17** because of inapplicability of classical activation agents, like thionyl and oxalyl chloride. Finally, we found an alternative TPP/TCA system which was used for the synthesis of the both C-6-(alkylcarboxamide)-linked LEV "monomers" and "dimers" **17**, **18**.

A range of CIP derivatives and LEV with different chain lengths were also evaluated for their stability at PBS buffer at physiological pH = 7.4 and 37ºC for 6-7 days. All tested compounds showed significant stability towards hydrolysis (>93% of unhydrolyzed compound remained) under present conditions.

CHAPITRE III ÉVALUATION BIOLOGIQUE DES DÉRIVÉS SYNTHÉTISÉS

1. Introduction

Ce chapitre sera consacré à l'évaluation *in vitro* de l'activité biologique des dérivés de la CIP et de la LEV.

Tout d'abord, un criblage initial de l'activité antimicrobienne des dérivés synthétisés contre des souches à Gram-positif et à Gram-négatif sera décrit. Nous présenterons également les études concernant l'activité de certains dérivés contre les souches de *S. aureus* Méticilline Résistantes (SAMR) et MultiDrogues Résistantes (SAMDR) . Aux vues des résultats d'activités antimicrobiennes obtenues, nous avons essayé de réaliser une relation structure-activité pour les différents dérivés de la CIP et de la LEV testés.

Dans la deuxième partie de ce chapitre, l'activité antimycobactérienne des composés synthétisés sera présentée. Nous discuterons l'influence des modifications structurales sur l'activité contre *M. tuberculosis*.

La dernière partie de ce chapitre sera dédiée à l'évaluation de l'activité antiproliférative des dérivés de la CIP et de la LEV. Nous discuterons les résultats concernant leur cytotoxicité sur cinq lignées cancéreuses humaines. Nous mettrons en évidence l'influence des modifications structurales de la CIP et de la LEV sur leur potentialité et leur spectre d'activité cytotoxique.

En conclusion, nous donnerons un aperçu de l'ensemble des propriétés biologiques et nous essayerons de décrire les relations structure-activité générales concernant l'influence du type de substitution, de la nature du noyau quinolone et de la structure "monomère" ou "dimère" sur la sélectivité et la potentialité des dérivés contre ces cibles biologiques.

2. L'activité antibactérienne

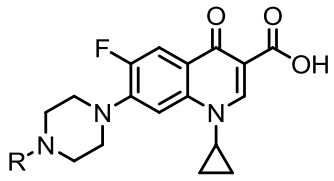
De part la propriété antibiotique majeure des composés parents, la CIP et LEV, les activités antimicrobiennes des composés synthétisés ont été déterminées. Cette étude a été effectuée par le Professeur Christine Roques au Laboratoire de Microbiologie Industrielle, UFR des Sciences Pharmaceutiques, Université Paul Sabatier, Toulouse.

Un premier criblage contre quatre souches standards de bactéries sélectionnées a été effectué. Cette étude a été réalisée sur deux souches Gram-négatifs : *E. coli* (bactérie impliquée dans la diarrhée et autres infections gastro-intestinales) et *P. aéruginosa* (bactérie impliquée dans les infections de blessures par brûlures et de l'oreille externe), ainsi que deux souches Gram-positifs : *S. aureus* (bactérie impliquée dans les infections de la peau et des plaies, les abcès, la pneumonie, la méningite ou l'ostéomyélite) et *Enterococcus hirae* (*E. hirae*, bactérie impliquée dans les infections de plaies). Les composés les plus actifs contre *S. aureus* ont été également testés sur un ensemble des souches SAMR et SAMDR qui représentent une cause majeure des maladies nosocomiales.

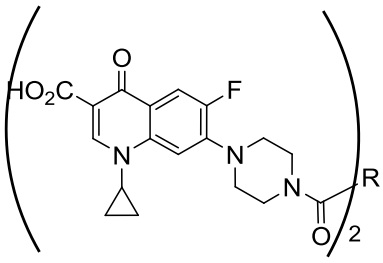
2.1 Activité antimicrobienne in vitro des dérivés de la CIP

Les concentrations minimales inhibitrices (CMI) et les concentrations minimales bactéricides (CMB) de certains composés synthétisés parmi les dérivés de la CIP ont été évaluées, comparativement à celles de la CIP (**Tableau 66**). La CMI est déterminée comme la concentration minimale de l'agent antibactérien qui inhibe la croissance visible de la souche. La CMB est déterminée comme la concentration minimale de l'agent antibactérien qui tue >99.9% de la souche. Ces concentrations sont déterminées par des techniques des dilution consécutives. On définit les antibiotiques comme bactéricides lorsque le rapport CMB/CMI est égal à 4, et les antibiotiques bactériostatiques, lorsque ce rapport est compris entre 8 et 16.

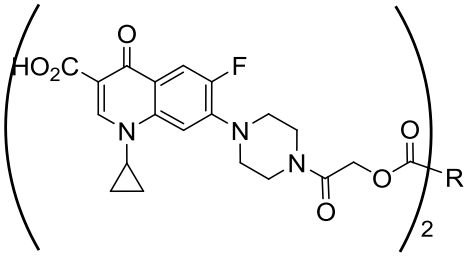
Tableau 66 Activité antimicrobienne *in vitro* des dérivés de la CIP



1, 2, 3, 4, 5, 6



7



9

			CMI (CMB), μM			
			Gram-négatif		*Gram-positif*	
Comp	*R*	*CLogP*[a]	*E. coli*[b]	*P. aéruginosa*[c]	*S. aureus*[d]	*E. hirae*[e]
CIP	H	-0,72	0,97(0,97)	0,97 (1,95)	0,97 (0,97)	0,97 (1,95)
2	$COCH_2Cl$	1,75	<0,97(<0,97)	27,8 (250)	<0,97 (<0,97)	3,9 (3,9)
1a	$COCH_3$	1,14	1,95 (1,95)	31,2 (31,2)	<0,97 (1,95)	7,8 (7,8)
1b	$COCH_2CH_3$	1,67	3,9 (>500)	31,2 (250)	<0,97 (<0,97)	3,9 (3,9)
1c	$CO(CH_2)_2CH_3$	2,19	7,8 (7,8)	62,5 (250)	<0,97 (<0,97)	7,8 (7,8)
1d	$COC(CH_3)_3$	2,37	15,6 (31,2)	62,5 (250)	<0,97 (<0,97)	7,8 (7,8)
1e	$CO(CH_2)_3CH_3$	2,72	7,8 (15,6)	62,5 (250)	<0,97 (<0,97)	7,8 (7,8)
1f	$CO(CH_2)_5CH_3$	3,78	31,2 (31,2)	62,5 (250)	<0,97 (<0,97)	31,2 (31,2)
1g	$CO(CH_2)_7CH_3$	4,84	31,2 (>500)	62,5 (250)	<0,97 (<0,97)	31,2 (31,2)
1h	$CO(CH_2)_8CH_3$	5,37	125 (>500)	125 (250)	7,8 (62,5)	15,6 (15,6)
1i	$CO(CH_2)_{10}CH_3$	6,43	7,8 (7,8)	62,5 (250)	3,9 (7,8)	7,8 (7,8)
1j	$CO(CH_2)_{12}CH_3$	7,48	125 (500)	125 (250)	125 (125)	62,5 (250)
1k	$CO(CH_2)_{14}CH_3$	8,54	125 (250)	125 (250)	125 (250)	62,5 (250)
1l	$COCH_2C_6H_5$	3,19	9,3 (7,8)	62,5 (250)	<0,97 (<0,97)	3,9 (7,8)
4	$COCH_2OH$	1,35	<0,97 (1,95)	15,6 (250)	<0,97 (1,95)	7,8 (7,8)
5	$COCH_2OCH_2Ph$	3,31	7.8 (15.6)	62,5 (250)	<0.97 (>500)	15,6 (62,5)
3a	$COCH_2OCOCH_3$	1,71	7,8 (15,6)	62,5 (250)	<0,97 (<0,97)	15,6 (15,6)
3b	$COCH_2OCO(CH_2)_2CH_3$	2,77	31,2 (31,2)	62,5 (250)	<0,97 (<0,97)	<0,97 (<0,97)
3c	$COCH_2OCO(CH_2)_4CH_3$	3,83	31,2 (31,2)	62,5 (250)	<0,97 (<0,97)	<0,97 (<0,97)
3d	$COCH_2OCO(CH_2)_6CH_3$	4,89	31,2 (62,5)	31,2 (250)	<0,97 (<0,97)	<0,97 (<0,97)
3e	$COCH_2OCO(CH_2)_7CH_3$	5,41	62,5 (500)	31,2 (250)	<0,97 (<0,97)	<0,97 (<0,97)
3f	$COCH_2OCO(CH_2)_8CH_3$	5,95	15,6 (15,6)	31,2 (250)	<0,97 (<0,97)	7,8 (15,6)

			CMI (CMB), μM			
			Gram-négatif		*Gram-positif*	
Comp	*R*	*CLogP*[a]	*E. coli*[b]	*P. aéruginosa*[c]	*S. aureus*[d]	*E. hirae*[e]
3g	$COCH_2OCO(CH_2)_9CH_3$	6,47	62,5 (250)	62,5 (250)	<0,97 (<0,97)	<0,97 (1,95)
3h	$COCH_2OCO(CH_2)_{10}CH_3$	7,00	125 (250)	62,5 (250)	<0,97 (<0,97)	7,8 (7,8)
3i	$COCH_2OCO(CH_2)_{12}CH_3$	8,06	125 (>500)	125 (250)	7,8 (7,8)	31,2 (31,2)
3j	$COCH_2OCO(CH_2)_{14}CH_3$	9,12	125 (250)	125 (250)	62,5 (500)	125 (500)
6a	$C(O)OCH_2CH_3$	2,82	7,8 (31,2)	62,5 (250)	<0,97 (<0,97)	3,9 (3,9)
6b	$C(O)O(CH_2)_3CH_3$	3,88	7,8 (31,2)	62,5 (250)	<0,97 (<0,97)	3,9 (7,8)
6c	$C(O)O(CH_2)_7CH_3$	5,99	125 (500)	125 (250)	7,8 (62,5)	125 (125)
6d	$C(O)O(CH_2)_8CH_3$	6,52	125 (500)	125 (250)	62,5 (125)	62,5 (250)
6e	$C(O)O(CH_2)_9CH_3$	7,05	125 (500)	125 (250)	62,5 (62,5)	62,5 (500)
6f	$C(O)O(CH_2)_{11}CH_3$	8,11	125 (125)	125 (250)	125 (125)	62,5 (>500)
6g	$C(O)OC(CH_3)_3$	3,53	1,95 (1,95)	15,6 (250)	<0,97 (<0,97)	15,6 (15,6)
7a	$(CH_2)_8$	n.d.	>250(>250)	62,5 (>125)	250 (<0, 48)	250 (250)
7b	$(CH_2)_{10}$	n.d.	62,5 (62,5)	62,5 (250)	<0,97 (<0,97)	125 (500)
7c	$(CH_2)_{12}$	n.d.	62,5 (500)	62,5 (250)	<0,97 (1,95)	125 (500)
7d	$(CH_2)_{13}$	n.d.	62,5 (500)	62,5 (250)	<0,97 (3,9)	125 (500)
7e	$(CH_2)_{14}$	n.d.	62,5 (500)	62,5 (250)	1,95 (15,6)	62,5 (500)
7f	$(CH_2)_{16}$	n.d.	125 (500)	125 (250)	125 (500)	125 (500)
9a	$(CH_2)_8$	n.d.	31,2 (62,5)	31,2 (125)	31,2 (>250)	62,5 (>250)
9b	$(CH_2)_{10}$	n.d.	31,2 (62,5)	62,5 (125)	< 0,48 (0,97)	31,2 (31,2)
9c	$(CH_2)_{12}$	n.d.	31,2 (62,5)	62,5 (125)	< 0,48 (<0,48)	15,6 (15,6)
9d	$(CH_2)_{13}$	n.d.	31,2 (62,5)	62,5 (250)	<0,97 (<0,97)	7,8 (15,6)
9e	$(CH_2)_{14}$	n.d.	7,8 (15,6)	62,5 (250)	<0,97 (<0,97)	31,2 (62,5)
9f	$(CH_2)_{16}$	n.d.	15,6 (31,2)	62,5 (250)	7,8 (15,6)	62,5 (125)

[a]La lipophilie (CLogP) a été évaluée par un calcul *in silico* avec le logiciel (ChemDraw Ultra 10,0) [b]*E. coli* CIP 53126 [c]*P. aeruginosa* CIP 82118 [d]*S. aureus* CIP 4.83 [e]*E. hirae* CIP 5855.

Quelle que soit la souche testée, les composés actifs (CMI ≤ 7,8 μM) présentent tous un rapport CMB / CMI inférieur à 4 indiquant que ce sont des bactéricides comme la CIP.

D'une manière générale, les activités inhibitrices et bactéricides des composés évalués contre la souche Gram-positif *S. aureus* ont été conservées. Parmi les 45 composés testés, l'activité antibactérienne *in vitro* pour 29 d'entre eux est équivalente à celle de la CIP (CMI: 0,97 μM) contre *S. aureus*. L'activité contre la souche Gram-positif *E. hirae* est plus aléatoire ; seuls 5 composés, appartenant à la série 7-(4-(2-oxoéthylalkanoate)pipérazin-1-yl) **3**, présentent la même activité antibactérienne *in vitro* comparé à la CIP.

En revanche, l'activité antibactérienne *in vitro* de tous les composés synthétisés sont 20 à 128 fois moins actifs contre *P. aeruginosa* et de 2 à 250 fois moins actifs contre *E. coli* que la CIP (CMI: 0,97 μM). Seuls les composés **2** (ClogP: 1,75) et **4** (1,35), qui sont les dérivés les plus polaires, montrent une activité antibactérienne *in vitro* contre *E. coli* équivalente à celle de la CIP.

Rappelons que le composé **4** est le produit issu d'une possible hydrolyse enzymatique ou chimique des dérivés de la série 7-(4-(2-oxoéthylalkanoate)pipérazin-1-yl) **3**. Le composé **4** est actif contre les deux souches Gram-positifs (*S. aureus* et *E. hirae)* avec des valeurs de CMI et CMB équivalentes à celles des dérivés **3a-l**. En revanche, alors que les dérivés **3a-j** ne sont pas actifs contre les deux souches Gram-négatifs (*E. coli* et *P. aeruginosa)*, le composé **4** présente une activité conte *E. coli* équivalente à celle de la CIP. Il semblerait donc que les dérivés de la série **3** ne subissent pas d'hydrolyse dans ces milieux cellulaires.

Une activité contre *S. aureus* comparable à celle de la CIP a été conservée :

- pour les dérivés de la CIP "monomères" 7-(4-(oxoéthylalcanoate) pipérazin-1-yl) **3a-h** possédant des valeurs de ClogPs comprises entre 1,71 et 7,00;
- pour les dérivés 7-(4-(alkyloxyoxycarbonyle)pipérazin-1-yl) **6a** (ClogP: 2,82); **6b** (3,88) et **6g** (3,53) ;
- pour les dérivés 7-(4-(alcanoyle)pipérazin-1-yl) **1a-g,k** (1,14<ClogP < 4,84).

Pour ces trois séries homogènes, les composés présentant les plus hautes valeurs de ClogP ne sont pas actifs contre *S. aureus.*

Parmi les dérivés "dimères" de la CIP **7a-f** et **9a-f**, les composés **7b, 9b ; 7c, 9c ; 7d, 9d** et **7e, 9e** sont aussi actifs ou deux fois moins actifs pour le dérivé **7e** (CMI: 1,95 μM) contre *S. aureus* par rapport à la CIP.

Les FQs sont connues pour traverser la membrane cytoplasmique des bactéries Gram-positifs par diffusion passive. Seules les formes non chargées de ces composés peuvent diffuser à travers la bicouche lipidique des membranes biologiques[45a, 181]. Les FQs pénètrent dans les bactéries Gram-négatifs par une voie essentiellement hydrophile grâce aux porines ou par une

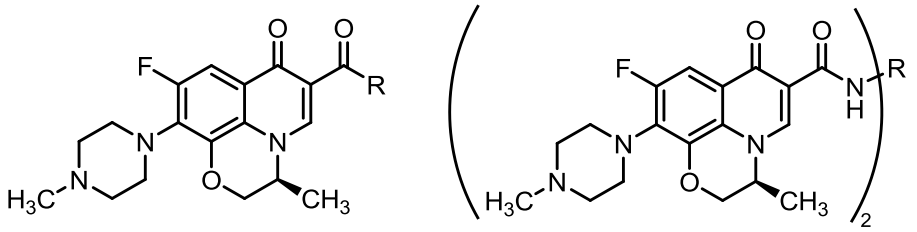
voie hydrophobe à travers la bicouche lipidique[182]. Il en découle que plus un médicament est hydrophile, plus la pénétration cellulaire se fera par la voie des porines alors que plus un médicament est hydrophobe, plus la diffusion au travers de la bicouche lipidique aura lieu.

La modification structurale de la CIP en position C-7 entraîne une rupture des propriétés zwittérioniques des dérivés synthétisés. Ainsi, pour ces dérivés de la CIP, seul l'équilibre non-chargé / déprotoné peut se produire, facilitant la diffusion passive à travers la bicouche lipidique, ce qui est cohérent avec les activités observées contre la souche Gram-positif *S. aureus* au détriment des souches Gram négatifs. Ces résultats sont en accord avec les travaux reportés dans la littérature par *Foroumadi et coll.*[67, 158, 183].

2.2 Activité antimicrobienne in vitro des dérivés de la LEV

Les résultats de l'évaluation des CMIs et des CMBs de certains dérivés de la LEV contre *S. aureus*, *E. hirae*, *P. aeruginosa* et *E. coli* ainsi que ceux de la LEV sont résumés dans le **Tableau 67**.

Tableau 67 Activité antimicrobienne *in vitro* des dérivés de la LEV



			CMI (CMB), μM			
			Gram-negatif		*Gram-positif*	
Comp	*R*	*CLogP*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. hirae*
LEV	OH	-0,51	0,97(0,97)	0,97 (0,97)	0,97 (0,97)	0,97 (0,97)
14d	$OCH_2OCO(CH_2)_8CH_3$	4,84	<0,97(<0,97)	< 0,97 (3,9)	< 0,97 (< 0,97)	0,97 (1,95)
14e	$OCH_2OCO(CH_2)_{10}CH_3$	5,90	3,9 (3.9)	< 0,97 (< 3,9)	< 0,97 (< 0,97)	1,95 (1,95)
14f	$OCH_2OCO(CH_2)_{13}CH_3$	6,96	15,6 (15,6)	62,5 (250)	7,8 (3,9)	15,6 (15,6)
17a	$NH(CH_2)_3CH_3$	2,13	62,5 (62,5)	62,5 (250)	125 (125)	125 (250)
17b	$NH(CH_2)_5CH_3$	3,19	125 (500)	62,5 (250)	250 (250)	125 (250)
17d	$NH(CH_2)_7CH_3$	4,25	125 (500)	62.5 (250)	31,2 (31,2)	31,2 (31,2)
17e	$NH(CH_2)_9CH_3$	5,31	125 (500)	62,5 (250)	7,8 (7,8)	7,8 (15,6)
17f	$NH(CH_2)_{11}CH_3$	6,36	125 (500)	62,5 (250)	3,9 (15,6)	3,9 (3,9)
17h	$NH(CH_2)_{13}CH_3$	7,42	125 (500)	62,5 (250)	7,8 (31,2)	3,9 (3,9)
17i	$NH(CH_2)_{14}CH_3$	7,95	125 (500)	62,5 (250)	15,6 (62,5)	7,8 (7,8)
18b	$(CH_2)_5$	n.d.	62,5 (62,5)	62,5 (250)	125 (500)	125 (250)
18c	$(CH_2)_6$	n.d.	62,5 (62,5)	62,5 (250)	125 (500)	125 (125)
18e	$(CH_2)_8$	n.d.	3,9 (3,9)	62,5 (250)	15,6 (15,6)	62,5 (125)
18f	$(CH_2)_9$	n.d.	3,9 (3,9)	62,5 (250)	31,2 (31,2)	31,2 (31,2)
18g	$(CH_2)_{10}$	n.d.	<0,97(<0,97)	31,2 (62,5)	7,8 (7,8)	31,2 (62,5)
18h	$(CH_2)_{12}$	n.d.	<0,97(<0,97)	31,2 (31,2)	1,95 (1,95)	3,9 (3,9)

As outlined in the introduction, the upper portion of the molecule which includes the C-3 carboxy and C-4 keto moieties of FQs is considered as essential for the antibacterial activity. Pyvaloyloxymethyl ester of ofloxacin, the racemate LEV, synthesized as prodrug by *Maeda et al.*[170] did not exhibit antibacterial activity against a panel of Gram-positive and Gram-negative strains (MIC > 25 μg/mL). Our previous report concerning the *in vitro* antimicrobial evaluation of various alkanoyloxymethyl esters of NAL ranging from 3 to 15 carbon units[167] showed that this chemical modification of the C-3 carboxylic group of NAL led to the loss of the antibacterial activity, as expected (CMI > 128 μg/mL). We however evaluated the *in vitro* antibacterial activity of 3 "monomer" alkanoyloxymethyl esters of LEV, **14d**, **14e** and **14f**, selected depending on their ClogPs which were included in the same range (from 4.84 to 6.96) than the most potent "monomer" CIP derivatives.

Surprisingly, these latter derivatives and in particular **14d** and **14e**, retained an antibacterial activity against the Gram-positive and Gram-negative standard strains. These activities could be due to the hydrolysis of the compounds into the parent LEV and therefore compounds could act as prodrugs. If hydrolyse occurs, this one should be more important as the alkyl chain length decreases and can explain that **14d** and **14e** are more potent against bacteria strains than **14f**. However, as we will discuss in next sections, acyloxymethyl ester derivatives **14a** (n=2)**, 14c** (n=6)**, 14d** (n=8) **and 14f** (n=12) showed IC_{50} and MIC value against *M. tuberculosis,* roughly similar to that of LEV whatever the chain length. Moreover, **14a** (n=2) exhibited an antiproliferative activity with IC_{50} values against cancer cell lines lower than those of LEV, whilst **14b** (n=4), **14c** (n=6) and **14d** (n=8) didn't inhibit the cancer cell lines growth. Compounds **14e-h** (10<n<16) displayed antiproliferative activities against U373-MG, LoVo and MCF-7 cancer cell lines with IC_{50} values for **14e** 200- to 2000-fold lower than that of LEV. So, these observations don't seem to be in favour of a hydrolyse for compounds **14e** and **14f**.

Some authors reported the synthesis and antimicrobial activities of carboxamide derivatives of LEV and CIP and showed that they displayed antibacterial activities, contradicting the dogma that C-3 carboxylic acid is required[85, 184]. In our case, the alkylcarboxamide "monomer" derivatives of LEV **17** were inactive against Gram-positive and Gram-negative strains. Compounds **17e-h** (5.31<ClogP<7.42) showed moderate activities against Gram-positive strains with MICs higher than LEV. But interestingly, when increasing the linker length of the *C*-6-(*N*-alkylcarboxamide)-linked LEV "dimers" **18,** an antibacterial activity in particularly against the Gram-negative *E. coli* strain was observed for compounds **18g-h**.

One time more, the active compounds (CMI ≤ 7.8 μM) are bactericides as their native parent, LEV, as they displayed a MBC / MIC ratio less than 4.

By considering “monomer” and “dimer” derivatives of LEV, for which the acidic function is modified, only the uncharged/protonated equilibria can occur. Even if the hydrophobicity of carboxamide “monomer” derivatives **17** allows them to diffuse through the membrane, they seem not to act effectively on the enzymatic target. The mechanism of antibacterial activity of *C*-6-(*N*-alkylcarboxamide)-linked LEV “dimers” **18** seem to differ as by increasing the alkyl chain length, not only the activity against Gram-positive strains increases but also the activity against Gram-negative strains. These results also show that amide function can act as a bioisostere of carboxylic acid and therefore new derivatives of FQ can be envisaged for the future as antibacterials.

2.3 In vitro antimicrobial activities of CIP derivatives against standard S. aureus, MRSA and MDRSA strains

Due to the observed *in vitro* activities against *S. aureus* of CIP derivatives, we selected the most potent derivatives to be tested against a standard strain of *S. aureus* (CIP4.83), two MRSA strains (ATCC33591 and ATCC33592) and two clinical isolates MDRSA strains (MDRSA1 and MDRSA2). The MICs and the MBCs of all the 29 selected compounds against these different strains of *S. aureus* are shown in **Table 68** along with those of CIP.

Table 68 *In vitro* antimicrobial activity (MIC(MBC) in μM) of selected coumpounds against the *S. aureus* standard strain (*Sa* CIP 4.83), two MRSA strains (*Sa* ATCC33591 and *Sa* ATCC33592) and two MDRSA clinical isolates (*Sa* MDRSA1 and *Sa* MDRSA2)

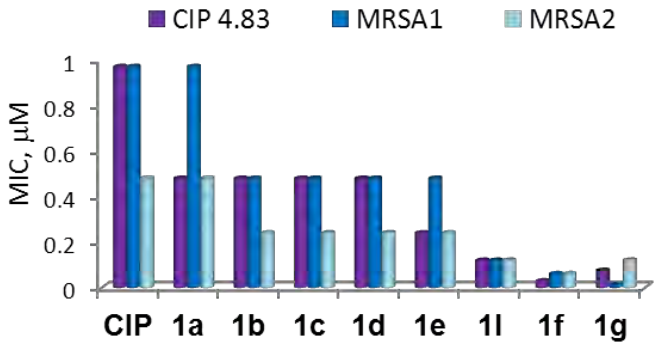
		MIC (MBC), μM				
		S. aureus strains			*Clinical isolates*	
Comp	*CLogP*[a]	*CIP 4.83*	*MRSA1*	*MRSA2*	*MDRSA1*	*MDRSA2*
CIP	-0.72	0.97 (1.95)	0.97 (1.95)	0.48 (0.97)	31.2 (62.5)	31.2 (31.2)
2	1.75	0.97 (1.95)	0.97 (0.97)	0.48 (0.97)	125 (500)	62.5 (62.5)
1a	1.14	0.48 (0.48)	0.97 (0.97)	0.48 (0.97)	62.5 (500)	31.2 (62.5)
1b	1.67	0.48 (0.97)	0.48 (0.97)	0.24 (0.48)	62.5 (500)	31.2 (31.2)
1c	2.19	0.48 (0.48)	0.48 (0.48)	0.24 (0.48)	31.2 (62.5)	31.2 (62.5)
1d	2.37	0.48 (0.97)	0.48 (0.48)	0.24 (0.48)	31.2 (500)	31.2 (31.2)
1e	2.72	0.24 (0.48)	0.48 (0.48)	0.24 (0.48)	62.5 (500)	31.2 (31.2)
1l	3.19	0.12 (0.24)	0.12 (0.24)	0.12 (0.24)	31.2 (62.5)	15.6 (15.6)
1f	3.78	0.03 (0.12)	0.061 (0.48)	0.061 (0.24)	62.5 (500)	250 (>500)
1g	4.84	$7.6\cdot10^{-3}$ (0.03)	0.015 (0.24)	0.12 (0.24)	31.2 (500)	31.2 (500)
3a	1.71	1.95 (3.9)	3.9 (3.9)	0.97 (1.95)	125 (500)	125 (125)
3b	2.77	0.24 (0.48)	0.48 (0.97)	0.061 (0.12)	15.6 (62.5)	15.6 (15.6)
3c	3.83	0.03 (0.24)	0.03 (0.061)	$3.8\cdot10^{-3}$ ($7.6\cdot10^{-3}$)	15.6 (500)	7.8 (15.6)
3d	4.89	$<9.5\cdot10^{-4}$ ($<9.5\cdot10^{-4}$)	$<9.5\cdot10^{-4}$ ($1.9\cdot10^{-3}$)	$<9.5\cdot10^{-4}$ ($<9.5\cdot10^{-4}$)	7.8 (500)	15.6 (15.6)
3e	5.41	$3.8\cdot10^{-3}$ (0.12)	0.03 (0.24)	0.015 (0.015)	7.8 (15.6)	7.8 (15.6)
3f	5.95	0.12 (0.97)	0.24 (0.48)	$3.8\cdot10^{-3}$ ($7.6\cdot10^{-3}$)	7.8 (15.6)	7.8 (15.6)
3g	6.47	0.97 (0.97)	0.97 (1.95)	$3.8\cdot10^{-3}$ (0.03)	15.6 (500)	7.8 (15.6)
3h	7.00	0.97 (1.95)	1.95 (3.9)	0.061 (0.97)	125 (500)	62.5 (250)
4	1.35	0.97 (1.95)	0.97 (1.95)	0.24 (1.95)	125 (500)	62.5 (62.5)
5	3.31	0.12 (0.24)	0.48 (0.97)	0.24 (0.97)	125 (500)	62.5 (125)
6a	2.82	0.97 (0.97)	0.97 (1.95)	0.48 (1.95)	125 (500)	62.5 (125)
6b	3.88	0.061 (0.12)	0.03 (0.12)	$7.6\cdot10^{-3}$ (0.12)	62.5 (500)	31.2 (31.2)
6g	3.53	0.12 (0.24)	0.061 (0.48)	0.12 (0.48)	31.2 (500)	15.6 (31.2)
7b	n.d.	0.12 (7.8)	0.48 (3.9)	0.12 (1.95)	125 (500)	125 (500)
7c	n.d.	0.12 (1.95)	0.061 (3.9)	0.12 (0.97)	7.8 (500)	7.8 (15.6)
7d	n.d.	0.061 (1.95)	0.061 (0.97)	0.061 (0.48)	1.95 (500)	15.6 (31.2)
9b	n.d.	1.95 (3.9)	3.9 (7.8)	0.48 (0.97)	250 (500)	125 (500)
9c	n.d.	1.95 (1.95)	1.95 (7.8)	0.015 (0.97)	125 (500)	62.5 (125)
9d	n.d.	0.12 (0.48)	0.48 (1.95)	0.12 (0.97)	62.5 (500)	31.2 (62.5)
9e	n.d.	1.95 (7.8)	7.8 (15.6)	0.97 (1.95)	125 (500)	125 (500)

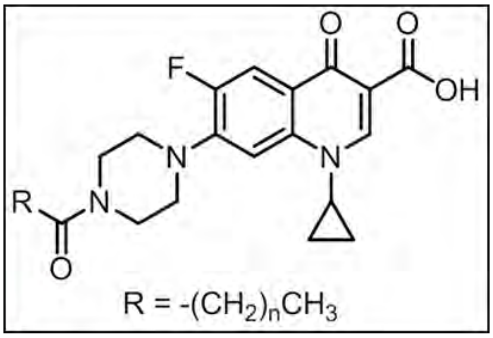
The first group of "mono-amide" derivatives displayed increased activity against both standard and methicillin-resistant strains. Compounds **1a-e,l** displayed MIC values somewhat equivalent with an improvement of MIC and MBC values towards standard, MRSA1 and MRSA2 strains than those of CIP. The most lipophilic (CLogPs = 3.78 and 4.84) compounds **1f,g** displayed interesting activities against standard and MRSA strains with a 60-fold decrease of MICs compared to CIP. However, none of compounds from the "mono-amide" group exhibited significant activity against MDR clinical isolates.

Considering the 7-(4-(alkanoyl)piperazin-1-yl) derivatives **1a-g** and **1l**, we observed a significant increase of activity only for two of them, that were compounds **1f** (ClogP = 3.78; M_w = 443.5 g·mol^{-1}; MICs from 0.03 to 0.061 μM) and **1g** (4.84 ; 471.6 g·mol^{-1}; 0.12 μM to 7.6 nM).

As shown in **Table 69**, representing the MICs values of **1a-g, 1l** homologous derivatives along with those of CIP against the standard CIP4.83 and the two MRSA strains, we observed a constant improvement of MIC and MBC values, comparing to CIP, for compounds **1b-e** and **1l** with ClogP values varying from 1.67 to 3.19. The two last and most lipophilic compounds **1f-g** (CLogPs = 3.78 and 4.84) turned out to be the most potent derivatives of this series.

Table 69 MICs values of CIP and homologous derivatives **1** against standard strains CIP4.83 and the two MRSA strains





Cmpd	**1a**	**1b**	**1c**	**1d**	**1e**	**1l**	**1f**	**1g**
n	0	1	2	-*	3	-*	5	7
CLogP	1.14	1.67	2.19	2.37	2.72	3.19	3.78	4.84
Mw, g·mol^{-1}	373.4	387.4	401.4	415.5	415.5	449.5	443.5	471.6

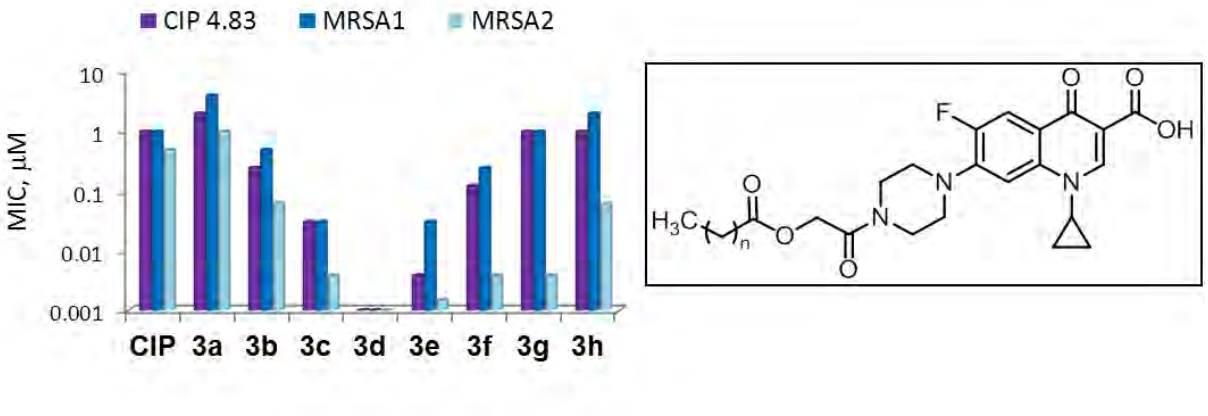
* R = *t*-Bu for **1d** and R = CH_2Ph for **1l**

Compounds **1f** (n = 5; cLogP = 3.78; Mw = 443.5 g.mol-1) and **1g** (7; 4.84; 471.6 g.mol-1) exhibited respectively MICs values 8- to 32- fold and 4- to 128- fold lower than those of CIP against standard CIP4.83 and the two MRSA strains.

The "mono-oxoethylester" CIP derivatives exhibited the highest degree of activity among all "monomeric" series. Compound **4**, resulting from a possible hydrolyse of derivatives **3**, displayed IC_{50} values equivalent to those of CIP. Once more, it seems that no hydrolyse of derivatives **3** occurs in bacterial cellular media.

The eight 7-(4-(oxoethylalkanoate)piperazin-1-yl) derivatives **3a-h** (2.77 < ClogPs < 5.95) displayed MICs and MBCs equivalent or lower than CIP. Among them, compounds **3d** (ClogP = 4.89; Mw = 515.6 g·mol^{-1}; MIC<0.95 nM) and **3e** (5.41 ; 529.6 g·mol^{-1}; from 0.03 μM to 3.8 nM) exhibited very good *in vitro* activities.

Table 70 MICs values of CIP and homologous derivatives **3** against standard strains CIP4.83 and the two MRSA strains



Cmpd	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**
n	0	2	4	6	7	8	9	10
CLogP	1.71	2.77	3.83	4.89	5.41	5.95	6.47	7.00
Mw, g·mol^{-1}	431.4	459.5	487.5	515.6	529.6	543.6	557.7	571.7

We observed a clear minimum on the curve representing MICs values of CIP and **3a-h** homologous derivatives against standard strains CIP4.83 and the two MRSA strains (**Table 70**) Compound **3d** (n = 6; cLogP = 4.89; Mw = 516.6 g.mol^{-1}), with subnanomolar MIC and MBC (< 0.95 nM) values against standard and methicillin-resistant strains, turned out to be the most potent derivatives with MICs values 500- to 1000- fold lower than those of CIP.

Among the 7-(4-(alkoxycarbonyl)piperazin-1-yl) derivatives **6**, only compound **6b** (n = 3; ClogP = 3.88; Mw = 431.5 g.mol^{-1}) exhibited lowest MICs than CIP and was found to be 16- to 63- fold more potent than CIP with a MIC value of 7.6 nM against MRSA strain ATCC33592.

In a general way, 7-(4-(alkanoyl)piperazin-1-yl))-linked CIP "dimers" **7**, displayed more potent activities against the standard and MRSA strains. Compound **7d** (n = 13) was found to be 8- to 16- fold more potent than CIP against these strains.

7-(4-(2-Oxoethylalkanoate)piperazin-1-yl))-linked CIP "dimers" **9** didn't show any significant enhancement of the activity against the standard and MRSA strains compared to CIP. Only compound **9d** (n = 13) was found to be 2- to 8- fold more potent than CIP.

For these two series of C7-C7-linked CIP "dimers", it would seem that the required conformation for an interaction with the enzymatic target is reached for a chain length of 13 carbon units.

On the other hand, even if some compounds exhibited better inhibitory activities against the two clinical isolates MDRSA (MDRSA1 and MDRSA2) than CIP, the benefit was less important.

No compounds from 7-(4-alkanoyl)piperazin-1-yl) **1**, 7-(4-alkoxycarbonyl)piperazin-1-yl) **6** and 7-(4-(2-oxoethylalkanoate)piperazin-1-yl))-linked **9** CIP derivatives were active against MDRSA strains.

7-(4-(Oxoethylalkanoate)piperazin-1-yl) derivatives **3b-g** ($2 < n < 9$; $2.77 <$ ClogPs < 6.47; $459.5 <$ Mw < 557.7 g.mol^{-1}) showed MICs from 7.8 to 15.6 μM against these two clinical isolates that is 2- to 4-fold more potent than CIP. From all series of tested "monomer" CIP derivatives, this series of derivatives **3** exhibited the more potent activities against the tested *S. aureus* strains.

7-(4-(Alkanoyl)piperazin-1-yl))-linked CIP "dimer" **7c** was found to be 4- fold more potent than CIP against the two clinical isolates with MICs of 7.8 μM. The most potent compound against MDRSA1 was found to be the "di-amide" derivative **7d** (MIC: 1.95 μM), that is 16- fold lower than that of CIP.

We can also notice that if active compounds against standard and MRSA strains retained a bactericide activity, this is not always true for MDRSA strains.

To conclude, for the most potent compounds against *S. aureus*, the activity is also maintained for MRSA strains. For each series of "monomer" derivatives the activity against standard and MRSA strains increases with ClogP until an optimal value of ClogP (**Tableau 66**, **Table 68**). We observed an optimal value for ClogP = 4.84 for the alkanoyl **1** CIP derivatives, 4.89 for the oxoethylalkanoate **3** and 3.88 for the alkoxycarbonyl **6**. The more potent antibacterial compounds were found in the oxoethylalkanoate **3** series, but whatever the considered

"monomer" series, all of them showed better activities against *S. aureus* strains than "dimer" CIP derivatives.

2.4 SAR attempts

Several general trends in antibacterial activity were deduced from these studies.

- Both active CIP or LEV derivatives ("monomeric" and "dimeric" structures) against bacterial strains (with CMIs values ≤ 7.8 μM) were bactericides (CMB / CMI < 4). So, they displayed the same antimicrobial profile than their native parents, CIP and LEV. It is nevertheless necessary to specify that this observation is not general for the antibacterial activities observed against MDRSA strains.
- Tested compounds were significantly less active towards Gram-negative species in comparison with the native parents CIP and LEV.
- Nevertheless, CIP and LEV derivatives didn't display the same antimicrobial profile of activity.
    - CIP derivatives showed a selectivity of the antimicrobial activity against *S. aureus*.
    - LEV derivatives showed various antimicrobial profile according to the structure.

2.4.1 SAR on CIP derivatives

The substitution of the distal nitrogen-4 atom at C-7 position of CIP resulted in an increased activity specifically towards *S. aureus* along with a significant decrease against other pathogens in comparison with the broad-spectrum activity of CIP.

The most active CIP derivatives against the various studied strains of *S. aureus* are represented in **Table 71**

The degree of activity is strongly dependent on the nature of attached functional group and decreases in the following order:

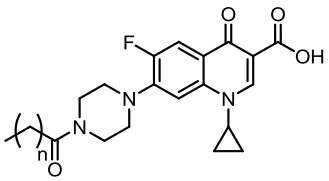
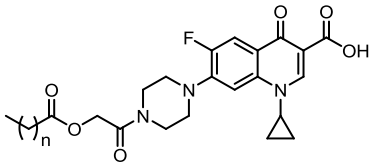
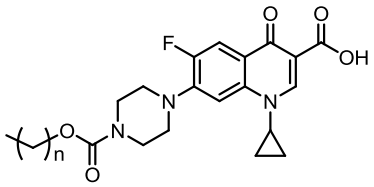
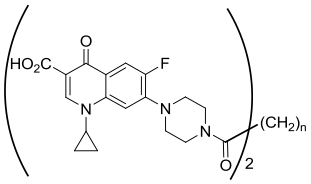
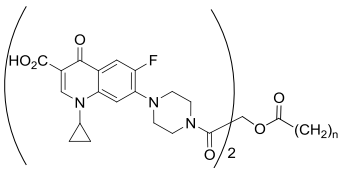
- for "monomers": oxoethylesters > amides ≈ carbamates,
- and the reversed order for "dimers": amides > oxoethylesters.

There is also a strong influence of the chain length on MIC values. The highest activity could be achieved with values of methylene groups of respectively n = 7 and 6 for "mono-amide" and "mono-oxoethylesters" derivatives. For these two series, the activities against *S.*

aureus evolve in the same way according to the chain length and the ClogP. As outlined before, too short or too long attached moieties was not beneficial. The optimal CLogP values were situated within a close range for the most active "monomers" **1g** and **3d** (CLogP = 4.84 and 4.89 respectively). These observations can't be done for the series of "mono-carbamates" derivatives **6** as the highest activity is achieved with a chain length of n = 3 and a ClogP = 3.88. In fact, no derivatives with a chain length included between n = 3 (ClogP = 3.88) and n = 7 (5.99) were synthesized.

Concerning the "dimer" CIP derivatives, it seems that the optimal chain length ranges around 12-13 methylene groups and that the presence of two quinolone cores in the molecule leads to an enhanced activity against MDRSA clinical isolates.

Table 71 Most active CIP derivatives against the various strains of *S. aureus*

			MIC, µM			
			S. aureus strains			
Structure	*Comp (n)*	*CLogP*	*CIP 4.83*	*MRSA1*	*MRSA2*	*MDRSA*
	**1f** (5)	3.78	0.03	0.061	0.061	Non active
	1g (7)	**4.84**	**$7.6.10^{-3}$**	**0.015**	**0.12**	Non active
	**3c** (4)	3.83	0.03	0.03	$3.8.10^{3}$	7.8-15.6
	3d (6)	**4.89**	**< $9.5.10^{-4}$**	**< $9.5.10^{-4}$**	**< $9.5.10^{-4}$**	7.8-15.6
	3e (7)	5.41	$3.8.10^{-3}$	0.03	0.015	7.8-7.8
	3f (8)	5.95	0.12	0.24	$3.8.10^{-3}$	7.8-7.8
	3g (9)	6.47	0.97	0.97	$3.8.10^{-3}$	7.8-15.6
	**6b (3)**	**3.88**	**0.061**	**0.03**	**$7.6.10^{-3}$**	Non active
	**7c (12)**	**Nd**	**0.12**	**0.061**	**0.12**	7.8-7.8
	7d (13)	**Nd**	**0.061**	**0.061**	**0.061**	**1.95**-15.6
	**9c** (12)	Nd	1.95	1.95	0.015	Non active
	9d (13)	**Nd**	**0.12**	**0.48**	**0.12**	Non active

2.4.2 SAR on LEV derivatives

Concerning LEV derivatives only preliminary screening against the four Gram-positive and Gram-negative standard strains were conducted.

In summary, the C-6 carboxyl group modification of LEV led to antibacterial profile and potency somewhat different than C-7 piperazine modification of CIP.

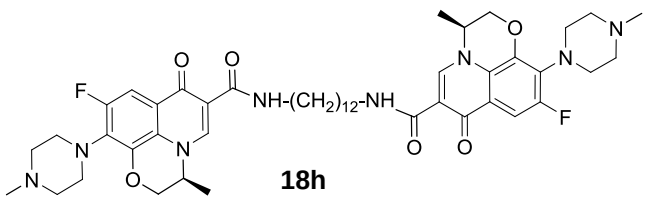
The degree of activity is also dependent on the nature of attached functional group and decreases in the following order:

- "mono-acyloxymethyl ester" derivatives **14** which displayed a broad spectrum of activity,
- "di-amides" derivatives **18** for which an antimicrobial activity was observed for compounds owning a chain length more than 10 methylene groups,
- "mono-amides" derivatives **17** were inactive.

Taking in account the possible hydrolysis of acyloxymethyl ester of LEV, it cannot be rational to conclude to a lead compound in this series without supplementary physico-chemical and biological evaluations.

The observed antibacterial activities of "di-amides" derivatives of LEV **18** can be due to the fact that amide function can act as a bioisostere of carboxylic acid for the formation of the drug-enzyme complex or to a modified mechanism of action of these compounds.

The C-6-(*N*-dodecylcarboxamide)-linked LEV "dimers" **18** seems to be the most promising compounds of all LEV derivatives for further antimicrobial studies (**Figure 46**).



18h

MIC *S. aureus*: 1.95 μM

MIC *E. hirae*: 3.9 μM

MIC *P. aeruginosa*: 31.2 μM

MIC *E. coli*: ≤0.97 μM

Figure 46 Lead compound of the series C-6-(*N*-acylcarboximide)-linked LEV "dimers"

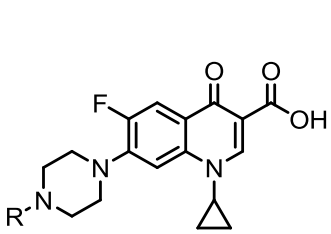
3. Antimycobacterial activity

We have also explored the effect of increasing the lipophilic character of CIP and LEV on activity against *M. Tuberculosis* ($H_{37}R_v$ strain) as penetration is crucial for FQ activity against this strain. The antimycobacterial activity of synthesized derivatives was evaluated by Patricia Constant at IPBS (Group of Dr. Mamadou Daffé, Institut de Pharmacologie et Biologie Structurale), Département Mécanismes Moléculaires des Infections Mycobactériennes, Toulouse.

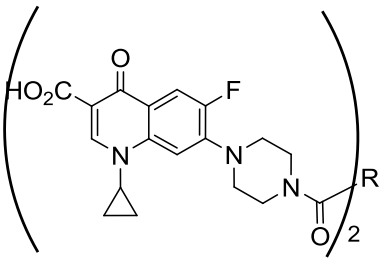
3.1 In vitro antimycobacterial inhibitory activities of CIP derivatives

The percentage of inhibition of *M. tuberculosis* growth was first determined for some selected compounds, along with those of CIP at concentrations of 10 and 1 μM and 0.1 μM. The percentage of inhibition of $H_{37}R_v$ obtained at 10 and 1 μM are summarized in**Table 72**.

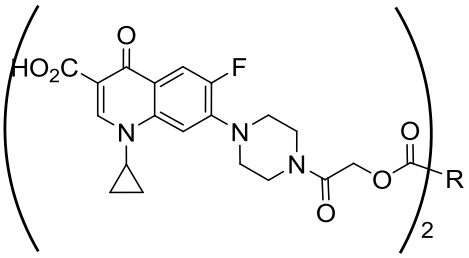
Table 72 Percentage of inhibition of *M. Tuberculosis* growth for selected CIP derivatives



1, 2, 3, 4, 5, 6



7



9

Comp	*R*	*CLogP*	*% inhibition of $H_{37}R_v$ at 10 μM*	*% inhibition of $H_{37}R_v$ at 1 μM*
CIP[a]	H	-0.72	98	95
2	$COCH_2Cl$	0.343	95	6
1a	$COCH_3$	1.14	92	0
1b	$COCH_2CH_3$	1.67	93	3
1c	$CO(CH_2)_2CH_3$	2.19	93	3
1d	$COC(CH_3)_3$	2.37	93	2
1e	$CO(CH_2)_3CH_3$	2.72	92	0
1f	$CO(CH_2)_5CH_3$	3.78	96	0

Comp	*R*	*CLogP*	*% inhibition of $H_{37}R_v$ at 10 μM*	*% inhibition of $H_{37}R_v$ at 1 μM*
1g	$CO(CH_2)_7CH_3$	4.84	95	2
1h[b]	$CO(CH_2)_8CH_3$	5.37	97	14
1i[b]	$CO(CH_2)_{10}CH_3$	6.43	96	16
1j	$CO(CH_2)_{12}CH_3$	7.48	47	3
1k	$CO(CH_2)_{14}CH_3$	8.54	14	0
1l	$COCH_2C_6H_5$	3.19	92	0
4	$COCH_2OH$	1.35	88	2
5	$COCH_2OCH_2Ph$	3.31	93	3
3c	$COCH_2OCO(CH_2)_4CH_3$	3.83	96	5
3d	$COCH_2OCO(CH_2)_6CH_3$	4.89	96	4
3f	$COCH_2OCO(CH_2)_8CH_3$	5.95	96	5
3g	$COCH_2OCO(CH_2)_9CH_3$	6.47	95	7
3h	$COCH_2OCO(CH_2)_{10}CH_3$	7.00	95	4
3i	$COCH_2OCO(CH_2)_{12}CH_3$	8.06	93	2
3j	$COCH_2OCO(CH_2)_{14}CH_3$	9.12	85	0
6a	$C(O)OCH_2CH_3$	2.82	92	0
6b	$C(O)O(CH_2)_3CH_3$	3.88	95	0
6c	$C(O)O(CH_2)_7CH_3$	5.99	96	7
6d	$C(O)O(CH_2)_8CH_3$	6.52	78	8
6e	$C(O)O(CH_2)_9CH_3$	7.05	71	7
6f	$C(O)O(CH_2)_{11}CH_3$	8.11	80	0
6g	$C(O)OC(CH_3)_3$	3.53	64	0
7a	$(CH_2)_8$	n.d.	0	0
7c	$(CH_2)_{12}$	n.d.	11	2
7d	$(CH_2)_{13}$	n.d.	40	5
7f	$(CH_2)_{16}$	n.d.	3	0
9a	$(CH_2)_8$	n.d.	62	0
9b	$(CH_2)_{10}$	n.d.	26	4
9c	$(CH_2)_{12}$	n.d.	87	6
9f	$(CH_2)_{16}$	n.d.	1.5	2

[a]MIC/IC_{50} values (μM) from the literature **0.16-12**[185]**/1.15**[185a] [b]IC_{50} values were evaluated for the most active **1h** (0.91/8.45) and **1i** (0.65/>10)

At 10 μM, several "monomer" CIP derivatives displayed a percentage of inhibition of $H_{37}R_v$ growth more than 95% comparable to that of CIP (98%).

Concerning the 7-(4-alkanoyl)piperazin-1-yl) derivatives **1**, the percentage of inhibition was optimal in a ClogP range from 3.78 (**1f**, n = 5, Mw = 463.5 $g.mol^{-1}$) to 6.43 (**1i**, n = 10, 513.7 $g.mol^{-1}$) and decreased for higher ClogP (**1j** and **1k**, owning longer chain length (n = 12 and 14 respectively).

Among the tested 7-(4-(oxoethylalkanoate)piperazin-1-yl) derivatives **3**, this optimum was reached for ClogP ranging from 3.83 (**3c**, n = 4, Mw = 487.5 $g.mol^{-1}$) to 8.06 (**3i**, n = 12, 599.7 $g.mol^{-1}$) and in a similar manner decreased for derivatives with longer chain length (**3j**, n = 14). The results obtained for compound **4** resulting from the possible hydrolysis of derivatives **3** showed once again that no hydrolysis occured in the cellular media.

7-(4-(Alkoxycarbonyl)piperazin-1-yl) derivatives **6** are less potent as only compounds **6b** (ClogP = 3.88: n = 3, Mw = 431.5 $g.mol^{-1}$) and **6c** (5.99; n = 7, 487.6 $g.mol^{-1}$) exhibited a percentage of inhibition ≥ 95%.

The more potent CIP derivatives from series **1** and **3** displayed the best percentages of inhibition of $H_{37}R_v$ growth for CLogP and molecular weight values situated within a relatively large range (3.78 < ClogP < 8.06 and 463.51 < Mw < 599.7 $g.mol^{-1}$) and for a range of chain length between 4 to 12 methylene groups. For the CIP derivatives from series **6,** these ranges of values were more limited (3.88 < ClogP < 5.99; 3 < n < 7; 431.5 < Mw < 487.6 $g.mol^{-1}$) and we observed a decrease of inhibition of $H_{37}R_v$ growth from a ClogP value of 6.52 (**6d**, n=8; Mw = 501.6 $g.mol^{-1}$).

It could be notice that the "dimer" derivatives **7** and **9** of CIP were few active or inactive as the highest percentages of inhibition of $H_{37}R_v$ growth for these two series were 40% (**7d**) and 87% (**9c**).

At 1 μM, most of the "monomer" and "dimer" CIP derivatives were inactive as only compounds **1h** and **1i** showed percentages of inhibition around 15% while CIP inhibited the mycobacterial growth at 95%.

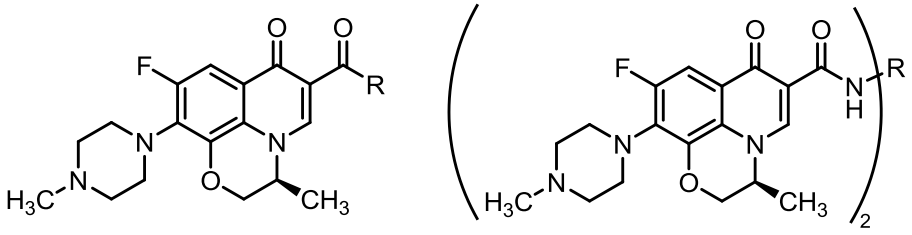
At 0.1 μM (data not shown), all compounds including CIP, didn't inhibit the mycobacterial growth.

This first screening has shown that increasing lipophilicity of CIP by a C-7 piperazine modification does not lead to an increase of the antimycobacterial activity as the more potent CIP derivatives are less potent than their native parent CIP at 1 μM.

3.2 In vitro antimycobacterial inhibitory activities of LEV derivatives

Derivatives of LEV were also evaluated for their inhibitory activity against *M. tuberculosis* at concentrations of 10, 1 and 0.1 μM along with those of LEV. These results are summarized in **Table 73**

Table 73 Percentage of inhibition of *M. Tuberculosis* growth for selected LEV derivatives



Comp	*R*	*CLogP*	*% inh. at 10 μM*	*% inh. at 1 μM*	*MIC, μM*	*IC_{50}, μM*
LEV[a]	OH	-0.51	**96**	**85**	**2.10** [b]	**0.78** [b]
14a	$OCH_2OCO(CH_2)_2CH_3$	1.67	96	74	3.33 [b]	1.60 [b]
14c	$OCH_2OCO(CH_2)_6CH_3$	3.79	96	89	3.33 [b]	1.40 [b]
14e	$OCH_2OCO(CH_2)_{10}CH_3$	5.68	97	89	3.33 [b]	1.26 [b]
14f	$OCH_2OCO(CH_2)_{12}CH_3$	6.74	96	6	2.50 [c]	1.50 [c]
14g	$OCH_2OCO(CH_2)_{14}CH_3$	7.79	95	0		
14i	$OCH_2OCO(CH_2)_{18}CH_3$	n.d.	0	0		
17b	$NH(CH_2)_5CH_3$	3.19	15	0		
17d	$NH(CH_2)_7CH_3$	4.25	98	0		
17e	$NH(CH_2)_9CH_3$	5.31	97	5	10	5.10 [c]
17g	$NH(CH_2)_{12}CH_3$	6.89	97	6	> 10	n.d.
17i	$NH(CH_2)_{14}CH_3$	7.95	96	6	10	4.00 [c]
17j	$NH(CH_2)_{16}CH_3$	9.00	93	3		
18b	$(CH_2)_5$	n.d.	77	5		
18c	$(CH_2)_6$	n.d.	60	0	>10	n.d
18f	$(CH_2)_9$	n.d.	98	20	5.00 [b]	1.96 [b]
18g	$(CH_2)_{10}$	n.d.	n.d.	n.d.	5.00 [c]	1.90 [c]
18h	$(CH_2)_{12}$	n.d.	n.d.	n.d.	2.50 [c]	1.60 [c]

[a] MIC/IC_{50} values from the literature **0.18-2.76**[185a, b, 185e, f] / **1.8**[185a, b] Mean of three experiments [c] Values from two different experiments

At 10 μM, several "monomer" LEV derivatives displayed a percentage of inhibition more than 95% comparable to that of LEV (96%).

Concerning the acyloxymethyl ester LEV derivatives, the percentage of inhibition was optimal in a ClogP range from 1.67 (**14a**, n = 2, Mw = 461.7 $g.mol^{-1}$) to 7.79 (**14g**, n = 14, 629.8 $g.mol^{-1}$) and the compound with the higher chain length (**14h,** n = 18, 685.9 $g.mol^{-1}$) did not inhibit at all the growth of *M. tuberculosis*.

Among the tested alkylcarboxamide LEV derivatives, this optimum was reached for ClogP ranging from 4.25 (**17d**, n = 7, Mw = 472.3 $g.mol^{-1}$) to 9.00 (**17j**, n = 16, 598.4 $g.mol^{-1}$).

The "dimer" alkylcarboxamide LEV derivatives **18f** (n = 9, Mw = 845.0 $g.mol^{-1}$) displayed a percentage of inhibition of the mycobacterial growth of 98%. Compounds with shorter chain length (**18b** and **18c**, n = 5 and 6) were less potent against *M. Tuberculosis*.

At 1 μM, only three compounds from the acyloxymethyl ester series, **14a** (74%)**, 14c** (89%) and **14e** (89%) displayed an activity similar to that of LEV (85%). However, the possibility of a hydrolysis of these compounds into the parent LEV remained even if this hydrolysis should be more important for **14a** (n = 2) than for **14c** (n = 6) or **14e** (n = 10).

At 0.1 μM (data not shown), all compounds including LEV, didn't inhibit the mycobacterial growth.

3.3 In vitro determination of MIC and IC_{50} against M. tuberculosis

This first screening permitted us to select some compounds for a determination of their MIC and/or IC_{50} values against *M. tuberculosis*. We have selected, for these tests, the most potent CIP and LEV derivatives at 10 and 1 μM.

For CIP derivatives, only two compounds were selected that were **1h** and **1i**. As we have seen, more LEV derivatives were active at 1 μM, so MIC and IC_{50} of several compounds of each series were evaluated including C-6-(*N*-acylcarboxamide)-linked LEV dimers **18g** and **18h** that were synthesized in the group after the evaluation of inhibitory activities. The results obtained for these various compounds are reported in **Table 72** and **Table 73** along with those of CIP and LEV.

These preliminary studies confirmed the observed results about the inhibition of *M. tuberculosis* growth, that were a more potent activity of derivatives of LEV than those of CIP. The MIC and IC_{50} values of CIP and LEV against $H_{37}R_v$ are in accordance with those of literature. Nevertheless, these studies are currently carried out as only one experiment had been done for some compounds and as derivatives **1h** and **1i** of CIP showed two very different values from the two separate experiments.

3.4 SAR on CIP derivatives

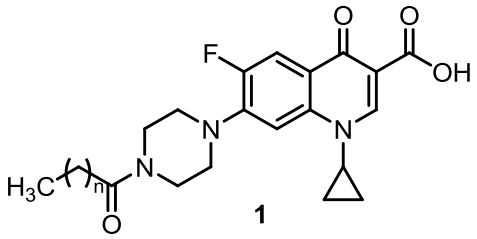
A SAR study is somewhat difficult to establish as, concerning the antimycobacterial activity, a C-7 modification of CIP was not beneficial as all "monomers" and "dimers" were less or as active as than their parent CIP. We also observed that "monomers" were much more active than the corresponding "dimers".

If we consider the "monomer" CIP derivatives we can conclude the following order of relative antimycobacterial activity: amides > oxoethylesters > carbamates.

The maximum percentages of inhibition of *M. tuberculosis* were observed for the most active compounds for whom ClogP values were included between 3.78 and 8.06, n between 4 and 12 and molecular weights between 463.5 and 599.7 $g.mol^{-1}$.

The most active compounds **1h** and **1i** belong to the amide series with ClogP values of 5.37 and 6.43; n = 8 and 10 and Mw = 485.6 and 513.7 $g.mol^{-1}$ (**Table 74**).

Table 74 The most active CIP derivatives against $H_{37}R_v$ strain



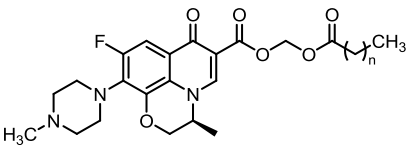
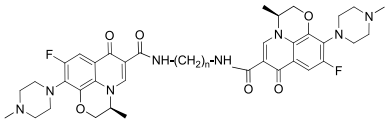
1

Comp	*n*	*CLogP*	*% inhibition at 10 μM*	*% inhibition at 1 μM*	*IC_{50}, μM*
1h	8	5.37	97	14	0.91/8.45
1i	10	6.43	96	16	0.65

1.1 SAR on LEV derivatives

A C-6 modification of LEV was more beneficial than C-7 modification of CIP with higher number of active compounds. The most potent derivatives of LEV, that were alkanoyloxymethyl esters LEV derivatives **14** and C-6(*N*-alkylcarboxamide)-linked LEV "dimers" **18** exhibited $IC_{50} \leq 2$ μM and MIC ≥ 2.5 μM. Alkylcarboxamide LEV derivatives **17** were the least active. Compound **14e** (**IC_{50} = 1.26 μM**; n = 10; ClogP = 5.68; Mw = 513.7 $g.mol^{-1}$) from alkanoyloxymethyl esters of LEV seems to be the more potent against *M. tuberculosis* among all "monomer" derivatives of LEV, while compound **18h** (**IC_{50} = 1.60 μM**; n = 12; Mw = 845.0 $g.mol^{-1}$) seems to be the more potent against *M. tuberculosis* among C-6(*N*-alkylcarboxamide)-linked LEV "dimers" (**Table 75**).

Table 75 The most active derivatives of LEV against $H_{37}R_v$ strain

Comp	*Ref*	*n*	*CLogP*	*% inhibition at 10 μM*	*% inhibition at 1 μM*	*IC_{50}, μM*
	**14a**	2	1.67	96	74	1.6
	14c	6	3.79	96	89	1.4
	14e	10	5.68	97	89	1.26
	14f	12	6.74	96	6	1.50
	**18f**	9	n.d.	98	20	1.9
	18g	10	n.d.	-	-	1.90
	18h	12	n.d.	-	-	1.60

4. Antiproliferative activity

The antiproliferative activity of synthesized compounds together with parent LEV and CIP was evaluated *in vitro* against five human cancer cell lines. For each of the compounds under study, nine concentrations were tested on two human apoptosis-resistant glioblastoma U373-MG[186], non-small cell lung cancer A549[187] cell lines and three apoptosis-sensitive prostate PC-3[188], colorectal LoVo[189], breast MCF-7[188] cancer cell lines. We made use of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, which indirectly assesses the effect of the potentially anticancer compounds on the overall growth of adherent cell lines[190]. The IC_{50} values, which are the concentrations that reduce the mean growth value of the cell lines by 50% (after having cultured the cells for 5 days in presence of the investigational compound), were determined for each drug and compared to the mean control growth value. The *in vivo* tolerance of some selected derivatives in mice was also determined as a maximum tolerated dose (MTD) which represents the highest single dose of the compound that can be administered acutely to healthy animals. This evaluation was conducted by Pr. Robert Kiss et al., at Laboratoire de Toxicologie, Institut de Pharmacie, Université Libre de Bruxelles, Belgium.

4.1 In vitro antiproliferative activities of CIP derivatives

4.1.1 In vitro antiproliferative activities of "monomer" derivatives

The IC_{50} values, which are the concentrations that reduce the mean growth value of the cells by 50%, were determined for each drug and compared to the mean control growth value and are listed in **Table 76.**

Table 76 *In vitro* antiproliferative activity of CIP "monomer" derivatives

			IC_{50} (μM)				
			Apoptosis-sensitive			*Apoptosis-resistant*	
Comp.	*n*	*CLogP*	*PC-3*	*LoVo*	*MCF-7*	*U373-MG*	*A549*
CIP	-	-0.72	143 ± 1	89 ± 2	476 ± 13	96 ± 4	280 ± 11
2	-	0.78	8 ± 0.1	17 ± 0.3	23 ± 0.2	4 ± 0.3	10 ± 0.2
1a	0	1.14	680 ± 33	323 ± 8	803 ± 15	535 ± 13	584 ± 14
1b	1	1.67	352 ± 7	306 ± 6	727 ± 17	279 ± 6	402 ± 21
1c	2	2.19	85 ± 2	148 ± 2	172 ± 9	104 ± 2	697 ± 18
1d	-	2.37	246 ± 4	93 ± 5	85 ± 1	67±3	509 ± 11
1e	3	2.72	73 ± 0.7	87 ± 2	316 ± 11	86 ± 2	64 ± 0.8
1f	5	3.78	779 ± 21	793 ± 14	754 ± 12	729 ± 9	808 ± 12
1g	7	4.84	7 ± 0.1	30 ± 0.4	26 ± 0.3	24 ± 0.3	6 ± 0.1
1h	8	5.37	4 ± 0.1	7 ± 0.2	20 ± 0.4	5 ± 0.2	3 ± 0.1
1i	10	6.43	4 ± 0.1	45 ± 0.9	108 ± 2	18 ± 0.2	7 ± 0.1
1j	12	7.48	94 ± 1	101 ± 0.9	96 ± 1	205 ± 3	56 ± 0.4
1k	14	8.54	114 ± 0.6	290 ± 4	145 ± 1	16 ± 0.1	65 ± 0.7
1l	-	3.19	243 ± 2	41 ± 0.6	32 ± 0.5	34 ± 0.7	29 ± 0.3
4	-	1.35	433 ± 19	408 ± 12	985 ± 26	294 ± 4	456 ± 8
5	-	3.31	692	96	242	85	82
3a	0	1.71	176 ± 2	268 ± 4	279 ± 6	99 ± 2	373 ± 8
3b	2	2.77	715 ± 10	613 ± 6	1000 ± 11	586 ± 6	456 ± 5
3c	4	3.83	14 ± 0.1	27 ± 0.8	72 ± 2	28 ± 0.7	20 ± 0.3
3d	6	4.89	23 ± 0.3	29 ± 0.4	818 ± 17	53 ± 0.7	16 ± 0.2
3e	7	5.41	257 ± 4	267 ± 2	255 ± 3	272 ± 7	216 ± 1
3f	8	5.95	416 ± 8	317 ± 2	353 ± 4	291 ± 5	273 ± 4
3g	9	6.47	nd[a]	828 ± 13	816 ± 17	>1000	>1000
3h	10	7.00	3 ± 0.1	39 ± 0.7	621 ± 12	49 ± 2	15 ± 0.2
3i	12	8.06	745 ± 14	766 ± 12	828 ± 14	748 ± 23	742 ± 18
3j	14	9.12	30 ± 0.4	75 ± 2	56 ± 0.8	119 ± 0.6	112 ± 1
6a	1	2.34	385 ± 14	272 ± 8	455 ± 12	228 ± 3	>1000
6b	3	3.40	9 ± 0.3	52±1	14 ± 0.2	31 ± 0.5	42 ± 1.3
6c	7	5.52	24 ± 0.6	43 ± 1	23 ± 1	79 ± 0.7	21 ± 0.5
6d	8	6.05	30 ± 0.5	30 ± 0.1	35 ± 2	283 ± 9	38 ± 2
6e	9	6.58	24 ± 0.7	32 ± 0.1	20 ± 1	401 ± 44	44 ± 5
6f	11	7.63	217 ± 2	347 ± 6	200 ± 3	94 ± 7	273 ± 7
6g	-	3.06	26 ± 0.6	40 ± 0.4	13 ± 0.3	35 ± 0.9	18 ± 0.2

[a]Not determined

In a general way, no clear correlation between CLogP values or chain length and *in vitro* cytotoxic activity was observed for "monomers" CIP derivatives.

The synthesized 7-(4-(substituted)piperazin-1-yl) derivatives and the native pharmacophore CIP, evaluated *in vitro* for their antitumor activity, displayed IC_{50} values ranging from μM to mM concentrations.

Compound **2** (*N*-(chloroacetyl)-CIP; ClogP = 0.78; Mw = 407.8 $g.mol^{-1}$) showed higher antitumor activity *in vitro* than CIP with mean IC_{50} values of 9 μM (mean IC_{50} values = 250 μM for CIP) and exhibited IC_{50} values 5- to 28-fold lower than that of CIP depending on human cancer cell lines.

Among the series of 7-(4-(alkanoyl)piperazin-1-yl) CIP derivatives (**1a-l)** with different chain lengths from one to fifteen carbon atoms (ClogP ranging from 1.14 to 8.54), only three compounds (**1g** (ClogP = 4.84; n = 7; Mw = 471.6 $g.mol^{-1}$; mean IC_{50}; 15 μM), **1h** (5.37; 8; 485.6 $g.mol^{-1}$; 5 μM) and **1i** (6.43; 10; 471.6 $g.mol^{-1}$; 10 μM)) showed ICs_{50} ≤15 μM (**Table 76**). The most potent compound against all cancer cell lines was **1h**, which exhibited IC_{50} values from 13- to 93-fold lower than that of CIP.

Among the series of 7-(4-(2-oxoethylalcanoate)piperazin-1-yl) CIP derivatives (**3a-j**) with increasing lipophilicity of the alkyl chain bearing one to fifteen carbon atoms (ClogP ranging from 1.71 to 9.12), only three derivatives (**3c** (ClogP = 3.83; n = 4; Mw = 487.5 $g.mol^{-1}$; mean IC_{50}; 26 μM); **3d** (4.89; 6; 515.6 $g.mol^{-1}$; 32 μM); **3h** (7.00; 10; 571.4 $g.mol^{-1}$; 37 μM)) showed higher activities than CIP but none of them showed higher activity when compared to compounds from the "mono-amide" series **1**. The most interesting compound of this series was **3h** whose IC_{50} value on the prostate cancer cell line (3 μM) was 47-fold lower than that of CIP. Stability tests of some compounds from series **3** in phosphate buffer at pH 7.4 have shown no degradation into **4.** As compound **4** had a very weak *in vitro* antitumor activity (mean IC_{50}: 484 μM), the observed antitumor activities recorded for **3c**, **3d** and **3h** are thus not related to a degradation of these compounds.

Compounds from the series of 7-(4-(alkoxycarbonyl)piperazin-1-yl) CIP derivatives (**6a-g**) with different length chains from two to twelve carbon atoms (ClogP ranging from 2.34 to 7.63) showed few or no *in vitro* antitumor activities. Indeed, compounds **6b-e, g** ($3.40 < ClogP < 6.58$; $1 < n < 9$; $431.46 < Mw < 515.62$ $g.mol^{-1}$; $33 <$ mean $IC_{50} < 26$ μM) presented *in vitro* antitumor activity against the five cancer cell lines that is weaker than series **1** and **3**. The most active compound **6b** (IC_{50} = 9 μM for PC-3) with a *n-Butyl* chain displayed IC_{50} values somewhat similar than its analog **6g** with a *t-Butyl* moiety.

In summary, among the three series of "monomer" CIP derivatives, some compounds displayed increased *in vitro* antitumor activity in comparison to their parent CIP regardless the type of cancer cell line (both resistant and sensitive to apoptosis). No clear dependence between cLogP and cytotoxicity was observed since the most active compounds **2** and **1h** greatly differed with their cLogP values (0.78 and 4.40 respectively). However, there was a strong influence of the type of attached group on the potency with the following order "amide" > "oxoethyl ester" ≈ "carbamate". We can conclude that the modification of the distal nitrogen at C-7 piperazine of CIP lead to more cytotoxic compounds with a homogenous activity profile for the two more potent derivatives.

4.1.2 In vivo maximum tolerated dose (MTD) in healthy mice

The *in vivo* tolerance of CIP, compound **2** and derivatives from series **1** and **6** with the lowest or highest *in vitro* activity (**1f, 1h**, **1k**, **6a**, **6b**, **6c** and **6g**) was determined as the MTD index, which represents the highest single dose of the compound that can be administered i.p. to experimental groups of three healthy mice per group over a maximum period of 28 days without causing their death[191]. All of which displayed MTD indices >80 mg/kg.

4.1.3 In vitro antiproliferative activities of "dimer" derivatives

C-7-((4-substitued)piperazin-1-yl)-linked CIP "dimers" were analogously evaluated in regards to their antiproliferative activity (**Table 77**).

Table 77 *In vitro* antiproliferative activity of CIP "dimer" derivatives

		IC_{50} (μM)				
		Apoptosis-sensitive			*Apoptosis-resistant*	
Comp.	*n*	*PC-3*	*LoVo*	*MCF-7*	*U373-MG*	*A549*
CIP	–	143	89	476	96	280
7a	8	350	339	826	358	423
7b	10	> 1000	> 1000	> 1000	141	> 1000
7c	12	175	15	5	5	82
7d	13	246	88	81	60	249
7e	14	8	6	8	3	7
7f	16	69	29	28	48	43
9a	8	358	389	> 1000	291	428
9b	10	454	476	> 1000	71	680
9c	12	281	43	9	3	437
9d	13	734	41	694	78	125
9e	14	0.2	0.1	1.0	0.8	8.7
9f	16	785	514	> 1000	516	838

The same antiproliferative profile was obtained for "dimers" of CIP **7a-f** and **9a-f**.

The 7-(4-(alkanoyl)piperazin-1-yl)-linked CIP **7c** (n = 12) and **7e** (n = 14) and the 7-(4-(oxoethylalcanoate)piperazin-1-yl)-linked CIP **9c** (n = 12) and **9e** (n = 14) were the more potent derivatives of these two series (**Table 77**). Compounds **7e** and **9e** displayed $ICs_{50} \leq 10$ μM against all cancer cell lines, whereas compounds **7c** and **9c** showed an *in vitro* inhibition of the human apoptosis-resistant U373-MG (5 and 3 μM respectively) and apoptosis-sensitive MCF-7 (5 and 9 μM respectively) cancer cell lines. Compounds **7e** and **9e** exhibited respectively IC_{50} values from 3 to 8 μM and from 0.1 to 8.7 μM that are 15- to 40-fold and 32- to 890-fold lower than that of CIP**.** For other compounds, no pharmacological benefit, in term of *in vitro* antitumor activity, was observed compared to CIP. The "dimer" **9e** turned out to be the most potent compound among the C-7/C-7-linked CIP "dimers".

4.1.4 General conclusion on antiproliferative activity of CIP derivatives

Several of the novel CIP derivatives displayed higher *in vitro* antitumor activity than parent CIP. By increasing lipophilicity of CIP, we could expect an increase in the affinity of the compounds for the cell membrane and thus an easier penetration into the cell. However, if substitutions on the piperazinyl group of CIP can lead to an increase in antitumor activity, this is not dependent on the lipophilicity. In comparing each homologous series or each cancer cell line independently, no correlation between ClogP and IC_{50} values can be observed.

However, in comparing the same chain length but with a different functional linker to CIP, the "monomer" alkanoyl derivatives **1** seem to be more potent than the alkoxycarbonyl **6** or oxoethylalcanoate **3** derivatives. For example, the most potent antiproliferative derivative **1h**, with a chain length of nine carbon atoms, is more active than the corresponding alkoxycarbonyl **6d** or oxyethylalcanoate **3f** derivatives.

In a general way, piperazinyl-linked CIP "dimers" displayed more potent antiproliferative effect than the monomers derivatives. For the most interesting "dimer" derivative **9e**, the two CIP moities are tethered by an oxoethylalcanoate linker whereas for the most interesting monomer derivative **1h**, an alkanoyl functionnal group is linked to CIP.

In comparing "mono-amide" versus "di-amide" series, the most active "mono-amide" **1h** (n = 8) showed the same homogeneous antiproliferative profile than the most active "di-amide" derivative **7e** (n = 14) (**Figure 47**, **A**)

In comparing "mono-oxoethylester" versus "di-oxoethylester" series, the most active "mono-oxoethylester" derivatives (**3c** (n = 4); **3d** (n = 6) and **3h** (n = 10)) showed a low activity

against all cancer cell lines while the most active "di-oxoethylester" derivative **9e** (n = 14) displayed a homogeneous antiproliferative profile with IC_{50} values < 10 µM (**Figure 47**, **B**).

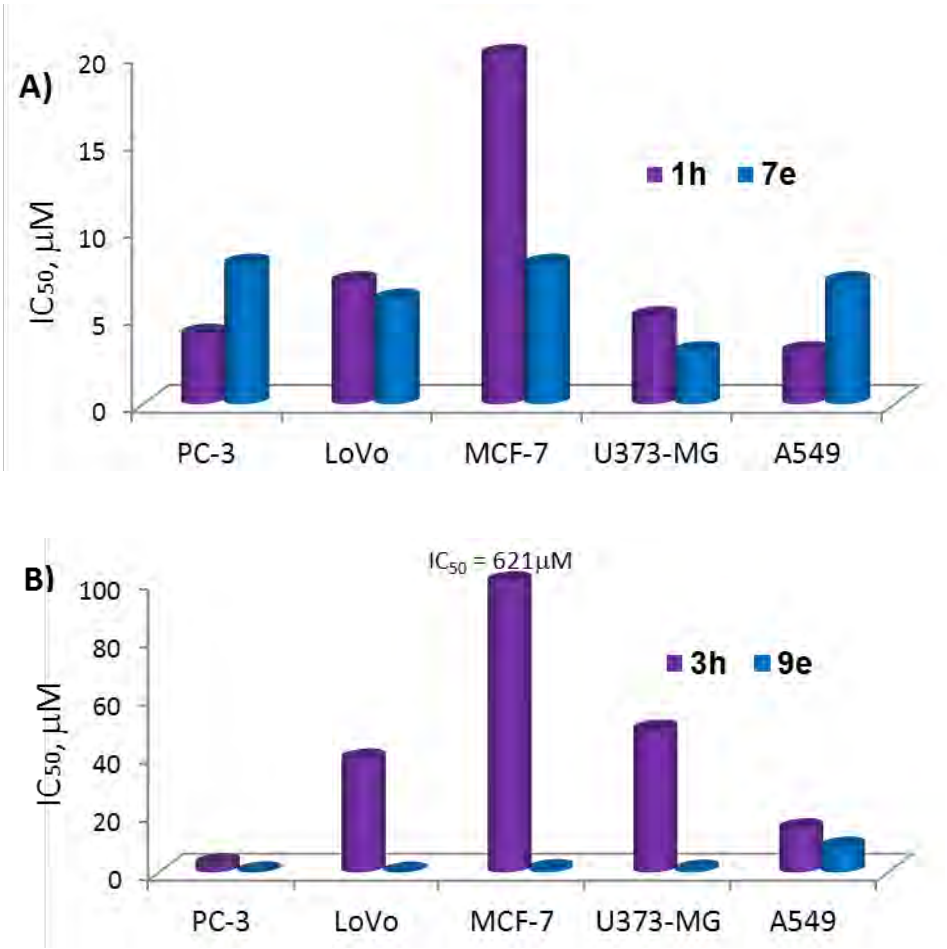


Figure 47 IC_{50} values (µM) of "monomer" and "dimer" CIP derivatives (A) : "mono-amide" **1h** versus "di-amide" **7e** CIP derivatives. (B) "mono-oxoethylester" **3h** versus "di-oxoethylester" **9e** CIP derivatives

In conclusion, the antiproliferative activity of CIP derivatives decreases in the following order : "di-oxoethylester" > "di-amide" > "mono-amide" > "mono-carbamate" ~ "mono-oxoethylester".

The "di-oxoethylester" **9e** displayed the highest cytotoxic activity among all CIP derivatives.

4.2 In vitro antiproliferative activities of LEV derivatives

4.2.1 In vitro antiproliferative activities of "monomer" LEV derivatives

Some C-6 substituted LEV derivatives were tested for their antiproliferative activity against the panel of five cancer cell lines (**Table 78**).

Table 78 Antiproliferative activity of "monomer" LEV derivatives

			IC_{50} (μM)				
			Apoptosis-sensitive			*Apoptosis-resistant*	
Comp.	*n*	*CLogP*	*PC-3*	*LoVo*	*MCF-7*	*U373-MG*	*A549*
LEV	-	-0.51	238 ± 12	67 ± 5	622 ± 19	188 ± 13	70 ± 6
14a	2	1.67	31 ± 4	5 ± 1	35 ± 3	40 ± 3	3 ± 1
14b	4	2.73	207 ± 12	162 ± 15	183 ± 12	199 ± 22	235 ± 16
14c	6	3.79	230 ± 14	209 ± 12	238 ± 13	243 ± 15	289 ± 12
14d	8	4.84	>1000	>1000	>1000	>1000	>1000
14e	10	5.90	86 ± 6	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	65 ± 5
14f	12	6.96	71 ± 7	21 ± 3	6 ± 1	8 ± 2	617 ± 15
14g	14	8.02	56 ± 5	6 ± 1	8 ± 1	4 ± 1	46 ± 4
14h	16	nd	100 ± 6	4 ± 1	12 ± 2	0.9 ± 0.2	593 ± 34
17a	3	2.13	30 ± 2	24 ± 3	55 ± 4	113 ± 18	46 ± 1
17b	5	3.19	12 ± 2	5 ± 1	16 ± 2	4 ± 1	6 ± 1
17c	6	3.72	4 ± 1	1 ± 1	5 ± 1	3 ± 1	3 ± 1
17d	7	4.25	1.5 ± 0.2	0.8 ± 0.4	2.1 ± 0.2	2.3 ± 0.1	2.2 ± 0.1
17e	9	5.31	3 ± 1	3 ± 1	5 ± 1	3 ± 1	3 ± 1
17f	11	6.36	5 ± 1	3 ± 1	10 ± 1	5 ± 1	5 ± 1
17h	13	7.42	3 ± 1	3 ± 1	5 ± 1	3 ± 1	5 ± 1
17i	14	7.95	5 ± 1	6 ± 1	15 ± 2	7 ± 1	8 ± 1

Although the "mono-acyloxymethyl ester" derivatives **14** of increasing lipophilicity (ClogP ranging from 1.67 to 8.02) showed different antiproliferative effects depending on the substituent grafted on the carboxylic acid, there was no clear-cut relationship between in vitro antitumor activity and lipophilicity. Among the "mono-amide" derivatives **17** of increasing lipophilicity (ClogP ranging from 2.13 to 7.95), only the less lipophilic compound **17a** displayed IC_{50} values >10 μM; the introduction of a group with higher lipophilicity led to derivatives **17b-f, 17h-i** that exhibited IC_{50} values in the micromolar range.

Only three "mono-esters" (**14b**, **14c**, **14d**) possessed poor activity (IC_{50} >100 μM) against all cell lines (**Table 78**). As far as the A549 lung cell line is concerned, the "mono-esters" (with

the exception of **14a**) generally displayed poor growth inhibition or an effect comparable to that of parent LEV; the "mono-amides" **17** were more potent.

"Mono-ester" **14e** (n = 10) was the most active compound against U373-MG, LoVo and MCF-7 cell lines, with IC_{50} values below the micromolar range, that were 220-2000-fold lower than that of LEV. "Mono-amide" **17d** (n = 7) displayed IC_{50} values in the micromolar range, 30-150-fold lower than that of LEV, in PC-3 and A549 NSCLC cell lines, and turned out to be the most potent derivative in this series.

A difference in selectivity was observed between "mono-ester" and "mono-amide" series. For example, esters **14e-h** (5.90 < ClogP < 8.02; 10 < n < 16) displayed a growth inhibitory effect against both the apoptosis-resistant U373-MG and apoptosis-sensitive LoVo and MCF-7 cancer cell lines, whereas they were not active against both apoptosis-sensitive PC-3 and apoptosis-resistant A549 cell lines. In contrast, amides **17b-f, 17h-i** (3.19 < ClogP < 7.95; 5 < n < 14) produced similar effects irrespective of the cell lines.

As shown in **Figure 48,** the various cancer cell lines showed an heterogeneous profile of sensitivity to the lead ester **14e** in relation to apoptosis sensitivity versus resistance, while they all displayed similar sensitivity to the lead amide **17d**.

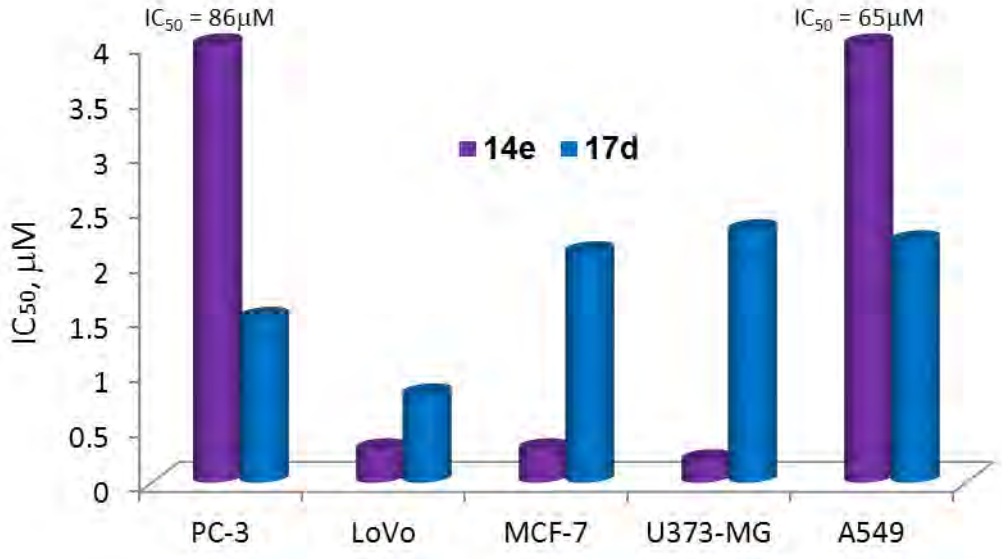


Figure 48 *In vitro* IC_{50} of compounds **14e** and **17d** with respect to apoptosis-resistant and sensitive cancer cell lines

In terms of antitumor activity, these data suggest that the "mono-amide" derivative **17d** was able to overcome the natural resistance of certain cancer cell types to apoptosis. The active derivatives under study could exert their antitumor activity through activation of apoptotic or non-apoptotic cell death processes.

Mondal et al.[192] and Yamakuchi et al.[193] evaluated the antiproliferative and apoptotic activities of LEV on human NSCLC and transitional bladder carcinoma cells in culture respectively. These investigators demonstrated that LEV caused cellular growth inhibition in a time-dependent manner. We also investigated the time course of the increase on growth inhibition of the stable "mono-amide" derivatives that exhibited similar antitumor effect against all the cell lines under study. In agreement with previous reports,[192-193] we observed significant increases in growth inhibition, on all tumor cell lines, from 3 to 5 days of exposure. For example, the cell number declines from 30-60% to 1-3% for the "mono-amide" **17d**, depending on the cell line (**Figure 49**).

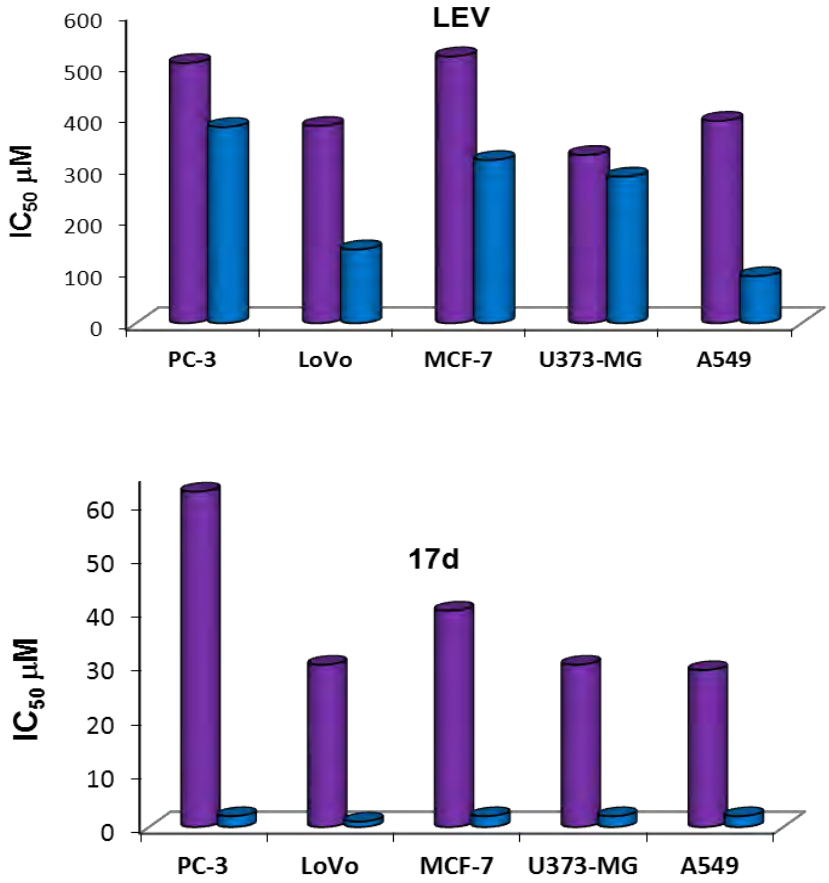


Figure 49 Time-dependent growth inhibition observed with LEV and "mono-amide" **17d** in human tumor cell. The cells were cultured for 3 (purple bars) or 5 (blue bars) days with compounds LEV or **17d**

4.2.2 In Vivo Maximum Tolerated Dose (MTD) in Healthy Mice

The *in vivo* tolerance of LEV and "mono-esters" **14** was determined as the MTD index, all of which displayed MTD indices >80 mg/kg.

4.2.3 In vitro antiproliferative activity of "dimer" LEV derivatives

IC_{50} values of "di-amide" derivatives along with those of LEV are summarized in **Table 79**.

Table 79 *In vitro* antiproliferative activity of LEV "di-amides"

		IC_{50} (μM)				
		Apoptosis-sensitive			*Apoptosis-resistant*	
Comp.	*n*	*PC-3*	*LoVo*	*MCF-7*	*U373-MG*	*A549*
LEV	–	238±12	67±5	622±19	188±13	70±6
18a	4	20 ± 2	19± 2	36 ± 2	30 ± 2	64.7 ± 1.5
18b	5	14 ± 2	9 ± 1	33 ± 5	23 ± 1	85 ± 13
18c	6	0.7 ± 0.1	1.2 ± 0.1	1.8± 0.1	2.4 ± 0.1	2.9 ± 0.2
18d	7	1.7 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	2.1 ± 0.1	2.3 ± 0.1
18e	8	1.6 ± 0.1	0.4 ± 0.1	1.8 ± 0.1	2 ± 60	2.2 ± 10
18f	9	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.7 ± 0.1
18g	10	120 ± 10	130 ± 20	360 ± 30	270 ± 10	480 ± 20
18h	12	2.8 ± 0.1	2.2 ± 0.1	3.8 ± 0.5	2.4 ± 0.1	4.5 ± 0.6

A homogeneous antiproliferative profile was obtained for *C*-6-(*N*-alkylcarboxamide)-linked LEV "dimers" **18a-h**. For each human cancer cell lines, there was a good correlation between the antiproliferative activities and the alkyl linker length. *C*-6-(*N*-butylcarboxamide)-linked LEV **18a** and *C*-6-(*N*-pentylcarboxamide)-linked LEV **18b** "dimers" exhibited $ICs_{50} \geq 9$ μM, whereas IC_{50} values were in the range from 0.2 to 4.5 μM against all cancer cell lines for compounds **18c-f, h** (chain length with $n \geq 6$). Thus, IC_{50} values decreased when the chain length increased, however we observed a break between *C*-6-(*N*-nonylcarboxamide)-linked LEV **18f** which exhibited the most potent antiproliferative activities against all cancer cell lines and *C*-6-(*N*-dodecanoylcarboxamide)-linked LEV **18h** as compound *C*-6-(*N*-decanoylcarboxamide)-linked LEV **18g** was inactive. These observed results led us to study the solubility of these 3 compounds (**18f**, **18g**, **18h**) in the cellular media for 24 hours at 37°C (**Figure 50**).

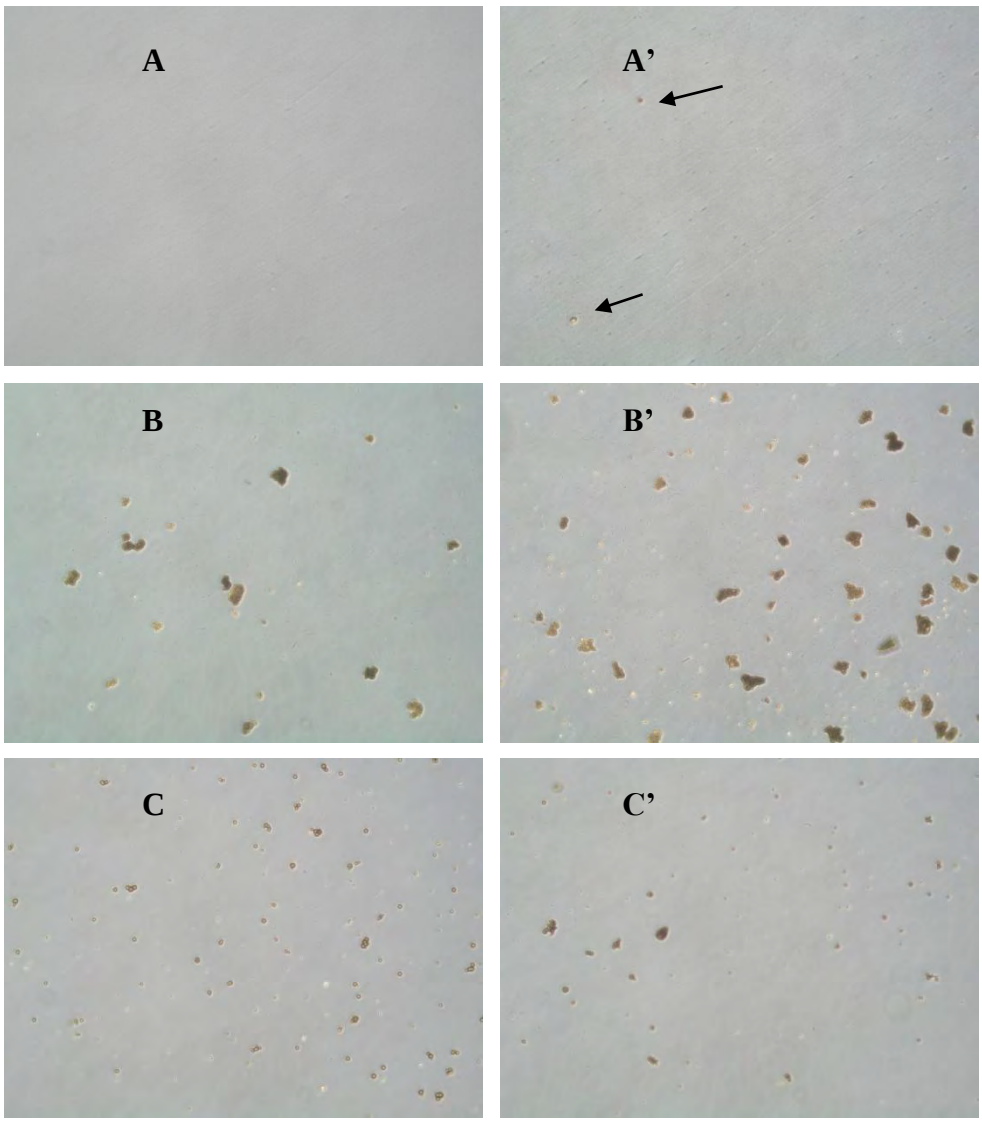


Figure 50 Solubility tests in cellular media for compound **18f** (n=9) at 5 μM (A, no precipitation) and 50 μM (A'), compound **18g** (n=10) at 50 μM (B) and 500 μM (B') and compound **18h** (n=12) at 5 μM (C) and 50 μM (C')

Compound **18f** didn't precipitate at concentrations of 5 and 50 μM while compound **18h** precipitated at 50 μM and also, but with less importance, at 5 μM. On the other hand, we observed a precipitation of compound **18g** at 500 or 50 μM. These results could explain why compound **18f** exhibited the most potent antiproliferative activities while there was a loss of activity for compound **18h** and no antiproliferative activity for compound **18g**. Nevertheless, the "dimer" **18f** exhibited the most potent activities against all cancer cell lines with IC_{50} values ranging from 0.2 to 0.7 μM, that is 100- to 1240-fold lower than that of LEV.

4.3 Conclusion on antiproliferative activity of LEV derivatives

We showed that "mono-amide" derivatives of LEV **17** exhibited better antiproliferative activities than the "mono-oxymethylester" **14.** The monomer (*N*-octyl)carboxamide derivative **17d** ($0.8 < ICs_{50} < 2.3$ μM) was more potent than the most interesting monomer dodecanoyloxymethyl ester derivative **14e** ($0.2 < ICs_{50} < 86$ μM). By analogy with C-6(*N*-alkylcarboxamide)-linked LEV "dimers" **18**, we also observed a decrease of the antiproliferative effect when the alkyl chain length was less than 6 methylene groups as the (*N*-butyl)carboxamide **17a** derivative of LEV was inactive ($24 < IC_{50} < 113$ μM). The alkylcarboxamide derivatives **17b-h** exhibited a homogeneous antiproliferative profile with IC_{50} values ranging from 0.8 to 16 μM against all the human cancer cell lines. On the other hand, alkanoyloxymethyl ester derivatives **14** exhibited a heterogeneous antiproliferative profile with ICs_{50} ranging from 0.2 to > 500 μM depending on the cancer cell lines. The "monomer" **17d** and the most potent antiproliferative "dimer" **18f** ($0.2 < ICs_{50} < 0.7$ μM), are both alkylcarboxamide derivatives of LEV.

Once more, "dimer" derivatives are more potent antiproliferative agents than "monomers" derivatives of LEV (**Figure 51**).

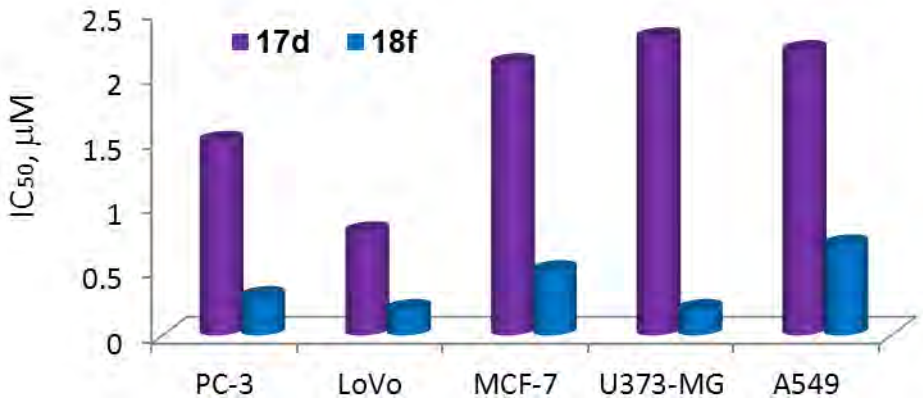


Figure 51 IC_{50} values (μM) of 'mono-amide" **17d** and "di-amide" **18f** derivatives of LEV

4.4 General conclusion on antiproliferative activity

This study thus has demonstrated that the cytotoxicity of CIP or LEV can be positively modulated through the introduction of simple substituents.

The derivatisation of C-6 LEV turned out to be more potent with regards to cytotoxic activity than C-7 modification of CIP. We can also notice the increased cytotoxicity of "dimer" compounds in comparison to "monomers" whatever the parent FQ. As we have previously noted

for the whole series of synthesized compounds we couldn't establish a clear-cut dependence between the chain length and the antiproliferative activity.

For "monomers" derivatives, we observed a profile of activity which was highly influenced by the functional group linked to FQ:

- The most active "mono-oxoethylester" CIP derivatives (**3c, 3d** or **3h**) and "mono-acyloxymethylester" derivatives of LEV (**14e-h**) displayed a heterogeneous antiproliferative profile of activity towards the different cancer cell lines. However, "mono-acyloxymethylester" derivatives of LEV were found to be more potent than "mono-oxoethylester" CIP derivatives.
- The most active "mono-amide" derivative of CIP **1h** and "mono-amide" derivatives of LEV (**17c-h**) both displayed homogeneous antiproliferative. Again, "mono-amide" derivatives of LEV were found to be more potent than "mono-amide" CIP derivatives.

Whatever the parent FQ, the dimerization of monomeric molecules led to an increase of the antiproliferative activity:

- The most active "di-amide" derivative of CIP **7e** was more potent than the most active "mono-amide" derivative of CIP **1h**. They both showed a homogeneous antiproliferative profile.
- The most active "di-oxoethylester" derivative of CIP **9e** was more potent than the most active "mono-oxoethylester" CIP derivatives (**3c, 3d** or **3h**). "Monomer" derivatives showed a heterogeneous antiproliferative profile while "dimer" derivatives showed a homogeneous one.
- The most active "di-amide" derivatives of LEV **18c-f, 18h** were more potent than the most active "mono-amide" derivatives of LEV **17c-h**. They both showed a homogeneous antiproliferative profile.

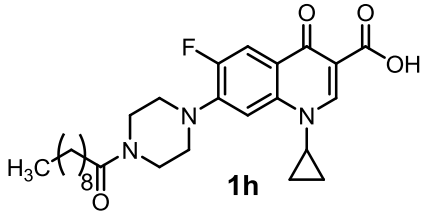
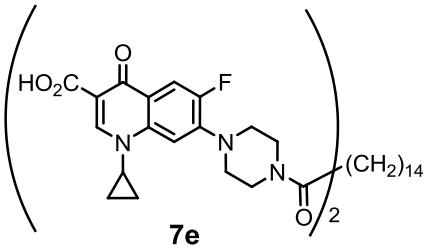
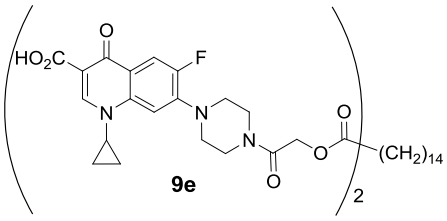
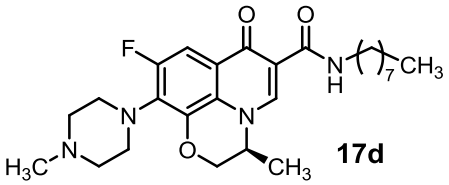
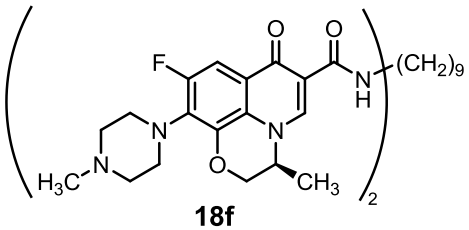
As seen in literature, the mechanisms of action by which some FQ antibiotics exert antitumor effects on various human cancer cell lines were determined to be through pro-apoptotic effects.

In terms of antitumor activity, these data suggest that the "amide" CIP derivatives and LEV and the "di-oxoethylester" derivative of CIP are able to overcome the natural resistance of certain cancer cell types to apoptosis as they all exhibited a homogeneous antiproliferative

profile. The active derivatives could exert their antitumor activity through activation of apoptotic or non-apoptotic cell death processes.

The most potent antiproliferative compounds are represented in **Table 80**.

Table 80 *In vitro* antiproliferative activity of the most active synthesized derivatives

	IC_{50} (μM)				
	Apoptosis-sensitive			*Apoptosis-resistant*	
Structure	*PC-3*	*LoVo*	*MCF-7*	*U373-MG*	*A549*
 **1h**	4	7	20	5	3
 **7e**	8	6	8	3	7
 **9e**	0.2	0.1	1.0	0.8	8.7
 **17d**	1.5	0.8	2.1	2.3	2.2
 **18f**	0.3	0.2	0.5	0.2	0.7

5. General overview of biological activity

The synthesized compounds displayed different level of specificity towards different biological targets.

Concerning the C-7 modification of CIP, it was beneficial with regards to antibacterial and antiproliferative activity while being less effective in the case of antimycobacterial potency. In a general way, "monomer" CIP derivatives were more potent as antibacterials against *S. aureus* while "dimers" were more potent as antiproliferative agents. "Monomer" derivatives showed a moderate activity against *M. tuberculosis* while "dimer" derivatives were inactive.

The antibacterial activity of "monomer" derivatives was optimal for a chain length ranging from n = 3 to 9 according to the linking group. Even if several "monomer" derivatives displayed antiproliferative activities greater than CIP, only the derivative **1h** (n = 8) showed significant IC_{50} values ranging from 3 to 20 μM depending on the cancer cell lines.

The antibacterial activity of "dimer" derivatives was optimal for n = 12 and mainly for n = 13 whatever the linking group while their antiproliferative activity was optimal for n = 12 and mainly n = 14. We could notice the most potent antibacterial compounds **7d** and **9d** (n = 13) were inactive against the cancer cell lines and that the most potent antiproliferative compounds **7e** and **9e** (n = 14) were few active against the *S. aureus* strain. (**Figure 52**).

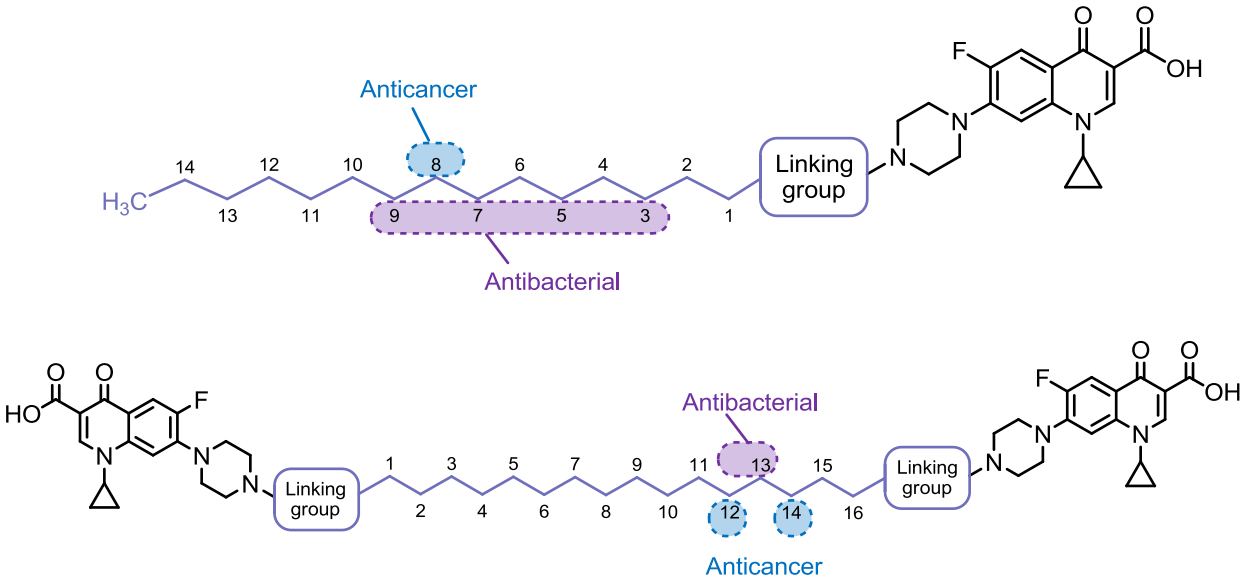


Figure 52 Influence of the chain length on the biological activity of CIP derivatives.

The C-6 modification of LEV was more beneficial with regards to cytotoxicity than for antibacterial and antimycobacterial activities.

The antibacterial activity of "mono-acyloxymethyl esters" derivatives was optimal for chain lengths equal to n = 8 (**14d**) and 10 (**14e**) while the antimycobacterial activity was optimal for n = 6 (**14c**) and 10 (**14e**). We could notice that these optimums were close for these two biological activities. On the other hand, the antiproliferative activity was optimal for longer chain lengths that were n = 12 (**14f**), 14 (**14g**) and 16 (**14h**) (Figure 47).

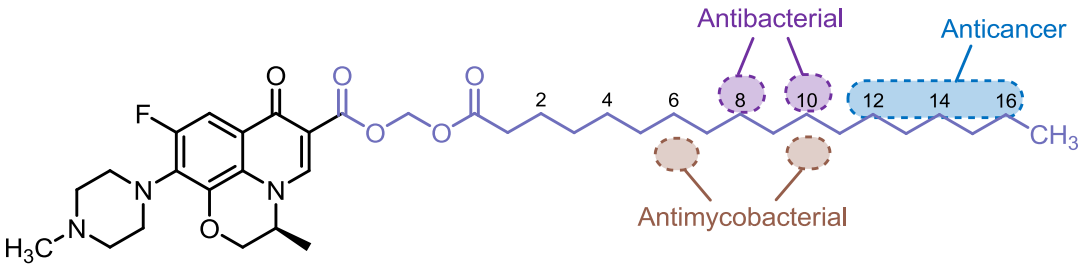


Figure 53 Influence of the chain length on the biological activity of LEV "mono-acyloxymethyl esters"

The introduction of an amide function at C-6 position of LEV led to a loss of activity of "mono-amide" derivatives against bacteria strains. The antimycobacterial activity was moderate as the most potent compound **17i** (n = 14) showed an IC_{50} value against *M. tuberculosis* of 4 µM, that is 5-fold less potent than LEV. On the other hand, a significant enhancement of the antiproliferative activity was observed for chain lengths ranging from n = 6 (**17c**) to n = 14 (**17i**) with a maximum of activity for n = 7 (**17d**) (**Figure 54**).

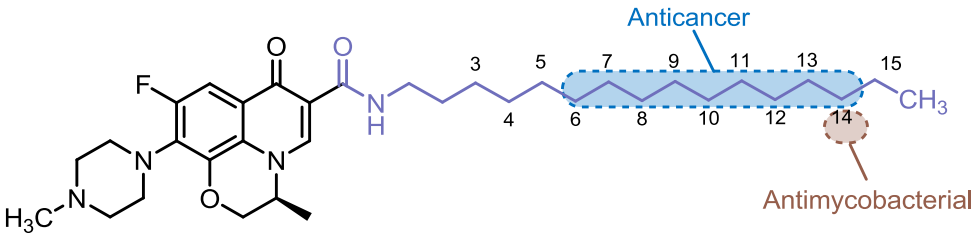


Figure 54 Influence of the chain length on the spectrum biological of activity for LEV "mono-amides"

"Di-amide" derivatives of LEV were the most interesting compounds whatever the biological activity. For compounds **18g** (n = 10) and mainly **18h** (n = 12), a broad spectrum of activity, as for the parent LEV, against Gram-positive and Gram-negative bacteria strains was

observed. A significant antimycobacterial activity was observed from n = 9 (**18f**) to n = 12 (**18h**) with IC_{50} values against *M. tuberculosis* < 2 μM. The best antiproliferative activities were observed for chain lengths ranging from n = 6 (**18c**) to n = 12 (**18h**) with an optimum for n = 9 (**18f**) (**Figure 55**).

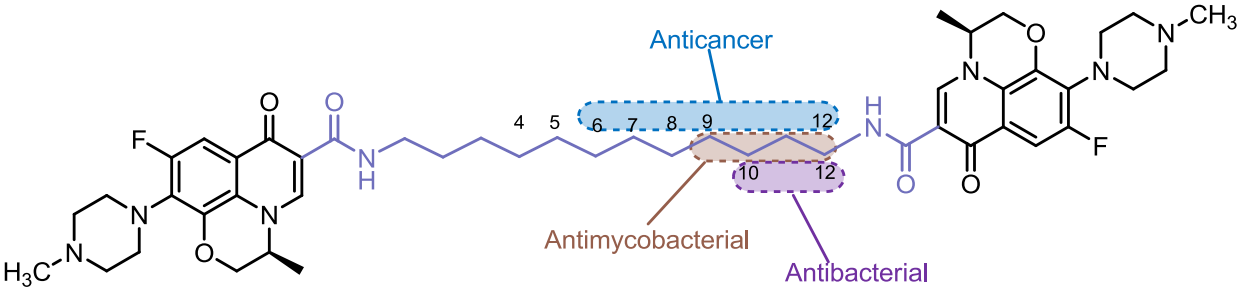


Figure 55 Influence of the chain length on the biological activity of LEV "di-amides"

Considering now the enzymatic target depending on the studied biological activity, remember that FQs corrupt the activities of DNA gyrase and topoisomerase IV. However, depending upon FQ employed and the bacterial species, either DNA gyrase or topoisomerase IV serves as the primary cytotoxic target of drug action. For most older FQ, including CIP and LEV, DNA gyrase in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria are often found to be the primary targets. Each quinolone drug appears to have a preferred target and the target selection can be altered by changes in quinolone structure. Moreover, *Kerns et al.* showed that DNA gyrase became the primary target for their synthetized "dimers" in Gram-positive *S. aureus* and *S. pneumoniae* strains[74-75].

DNA gyrase is the only type II topoisomerase present in *M. tuberculosis* and hence is the only target for FQ action.

FQs and their derivatives can exert their antitumor activity either by pro-apoptotic or through non-apoptotic-dependent mechanisms. As outlined in the introduction, FQs can act on human topoisomerase I or II and on several other biological targets.

We have reported in the following table (**Table 81**) the level of each biological activity for the most potent CIP derivatives and LEV against the Gram-positive *S. aureus* strain.

Table 81 Level of biological activity for selected most potent derivatives against *S. aureus* strain

			Antibacterial		*Antimycobacterial*	*Antiproliferative*
			Gram +	*Gram -*		
Postulated enzymatic target			*Topo IV > Gyrase*	*Gyrase > Topo IV*	*Gyrase*	*Topo I, Topo II*
Entry	*Compd.*	*ClogP (n)*				
1	**1f**	3.78 (5)	++[a]	-	+/-	-
2	**1g**	4.84 (7)	++	-	+/-	+
3	**3c**	3.83 (4)	++	-	+/-	+
4	**3d**	4.89 (6)	+++	-	+/-	+/-
5	**3e**	5.41 (7)	++	-	n.d.[b]	-
6	**3f**	5.95 (8)	++	-	+/-	-
7	**3g**	6.47 (9)	+	-	+/-	-
8	**7c**	n.d. (12)	++	-	-	+/-
9	**7d**	n.d. (13)	++	-	-	+/-
10	**14d**	4.84 (8)	+	+	n.d.	-
11	**14e**	5.90 (10)	+	+	++	++
12	**18g**	n.d. (10)	+/-	+	+	c
13	**18h**	n.d. (12)	+	+	+	++

[a] - : inactive +/- to +++ : from few active to more active than the parent CIP or LEV. [b] n.d. not determined. [c] problem of precipitation in the cellular media

Some trends can be extracted from this table:

- The more potent compounds from CIP derivatives (**Table 81**, **entries 1-9**) against the Gram-positive *S. aureus* strain are inactive against the other biological target.
- The more potent compounds from LEV derivatives (**Table 81**, **entries 10-13**) are generally less potent against the Gram-positive *S. aureus* strain than CIP derivatives. However, they showed an activity against Gram-negative strain and compounds **14e** (**entry 11**) and **18h** (**entry 13**) also displayed antimycobacterial and antiproliferative activities.
- The most potent compounds, from LEV series, against *M. Tuberculosis* are also active against Gram-negative strain. We can then suppose, that for these compounds, the enzymatic target could be DNA gyrase.

It would be so interesting to evaluate the IC_{50} values of these compounds against gyrase and topoisomerase IV from *S. aureus* and *E. coli*. So, we could determine if topoisomerase IV in Gram-positive and gyrase in Gram-negative remain to be the primary targets for the studied compounds. And mainly to determine if DNA gyrase didn't become the primary target for "di-amide" LEV derivatives **18g-h** (**Table 81, entries 12-13**) in Gram-positive strain.

In the next table (**Table 82**) are summarized the level of each biological activity for the most potent CIP derivatives and LEV against the cancer cell lines.

Table 82 Level of biological activity for selected most potent derivatives against cancer cell lines

			Antibacterial		*Antimycobacterial*	*Antiproliferative*
			Gram +	*Gram -*		
Postulated enzymatic target			*Topo IV > Gyrase*	*Gyrase > Topo IV*	*Gyrase*	*Topo I, Topo II*
Entry	*Compd.*	*ClogP (n)*				
1	**1h**	5.37 (8)	- [a]	-	+	++
2	**3h**	7.00 (10)	+/-	-	+/-	+
3	**7c**	n.d. (12)	++	-	-	+
4	**7e**	n.d. (14)	+/-	-	-	++
5	**9c**	n.d. (12)	+/-	-	+/-	+
6	**9e**	n.d. (14)	+/-	-	n.d. [b]	+++
7	**14e**	5.90 (10)	+	+	++	++
8	**14f**	6.96 (12)	-	-	+	+
9	**17d**	4.25 (7)	-	-	+/-	+++
10	**17e**	5.31 (9)	-	-	+/-	++
11	**17f**	6.36 (11)	+/-	-	n.d.	++
12	**17h**	7.42 (13)	+/-	-	n.d.	++
13	**17i**	7.95 (14)	-	-	+	++
14	**18c**	n.d. (6)	-	-	-	++
15	**18e**	n.d. (8)	-	+/-	n.d.	++
16	**18f**	n.d. (9)	-	+/-	+	+++
17	**18h**	n.d. (12)	+	+	+	++

[a]- : inactive +/- to +++ : from few active to more active than the parent CIP or LEV. [b]n.d. not determined.

Some trends can be extracted from this table:

- Except compound **7c** (**Table 82**, **entry 3**), no compound possessing an antibacterial activity showed an antiproliferative activity. Only "dimer" CIP derivatives **7e** and **9e** (**entries 4, 6**) displayed a good antiproliferative activity.
- More compounds from LEV derivatives (**Table 82**, **entries 7-17**) were found to have a good antiproliferative activity than an antibacterial one (**Table 82**, **entries 10-13**). Only LEV derivatives **14e** and **18h** showed these two biological activities.
- LEV derivatives are generally more potent as antiproliferative agents than CIP derivatives. However, we can notice that, except for compound **18h** (**entry 17**), they didn't display an antibacterial activity but some of them showed an antimycobacterial activity.

The mechanism of action by which these derivatives exert their antiproliferative activity will be more difficult to establish. In fact, the time- and dose-dependent pro-apoptotic effects of FQ were observed at higher doses than *in vitro* growth inhibition values. For example, it have been shown that most FQ required concentrations (IC_{50}s) to inhibit the human topoisomerase II of HeLa cells that were higher than those needed to inhibit bacterial type II topoisomerases. Moreover, no significant correlation was observed between the inhibition of HeLa cell topoisomerase II and that of *S. aureus* topoisomerase IV as well as *E. coli* DNA gyrase[194]. For CIP, the IC_{50} values to inhibit the topoisomerase II of Hela cells was found to be 3 and 478 higher than those needed to inhibit bacterial type II topoisomerases. So, the human polymerase II test can only be valuable if our compounds have IC_{50} values lower than CIP or LEV themselves.

So, mechanistic studies to investigate as whether our lead compounds induce either pro-apoptotic, pro-autophagic or cell proliferation inhibition should be envisaged. But, before proceeding with a genomic approach to identify the molecular pathways activated or inhibited by our compounds, we have to select a lead compound through the characterization of *in vivo* activity on a broad panel of biologically aggressive human xenograft models.

In summary, many CIP and LEV derivatives have been synthetized and evaluated for their biological activity. Some of these derivatives are non-toxic *in vivo* according to their MTD.

This study thus demonstrates that the biological activity of CIP and LEV could be positively modulated through the introduction of simple substituents on C-6 or C-7 positions.

Primary investigations have shown that a modification on the *N*-4 piperazinyl group of CIP can influence the biological activity: the main activity is specifically oriented against the

inhibition of *S. aureus* growth or oriented against the inhibition of cancer cell lines growth. No significant activity against *M. tuberculosis* was observed.

The most potent CIP derivatives against standard and MRSA strains displayed high MIC against standard *E. coli* strain and were inactive for the inhibition of human cancer cell lines growth. On the other hand, the most potent CIP derivatives as antiproliferative agents displayed moderate to poor activities as antibacterial agents.

The activity against *S. aureus* is greater for "monomeric" CIP derivatives as the antiproliferative effect is greater for "dimeric" ones.

A modification on the C-6 carboxylic group of LEV, and especially the substitution of this group by an alkylcarboxamide one, led to the most potent antiproliferative and antimycobacterial agents. So, we can conclude that C-6-carboxylic group of LEV is not required for the antiproliferative and antimycobacterial activities.

In a general way, the derivatives of LEV are less potent as antibacterial agents against *S. aureus* than CIP derivatives. However, some of them display an antibacterial activity against *E. coli.* For some "monomer" and "dimer" derivatives of LEV, the MICs values against *S. aureus* and *E. coli* are comparable to those of LEV, suggesting that this carboxylic acid modification is allowed for the antibacterial activity. These results could be useful for the development of new quinolone-based antibacterial agents.

One LEV derivative, that is *C*-6-(*N*-dodecanoylcarboxamide)-linked LEV **18h**, was found be be particulary interesting as it was the only one derivative showing a broad activity on the three biological target.

CONCLUSION GÉNÉRALE

En résumé, nous avons synthétisé par une procédure efficace cinq séries de dérivés “dimères” et “monomères” de la LEV et de la CIP. Nous avons mis au point de nouvelles conditions de réaction pour les dérivés “dimères” de la CIP parce que les conditions utilisées pour la synthèse des “monomères” de la CIP n’étaient pas efficaces et ont conduit à de faibles rendements. L’optimisation des conditions de réaction pour les "diesters" de CIP ont permis, avec l'utilisation de TBAI comme un catalyseur, de préparer ces composés avec de bons rendements. Les "di-amides" de CIP ont été obtenus en utilisant le carbodiimide EDCI soluble dans l’eau, ce qui a facilité le processus de purification. Les études les plus approfondies ont été menées au cours de la synthèse des "monoester" de la LEV lorsque les deux types de conditions par voie thermique et micro-ondes ont été évaluées. Après un examen détaillé de la réaction dans le cas des composés à chaîne courte et longue, nous avons obtenu une amélioration du rendement, un temps de réaction plus court et diminué la formation de sous-produit (conditions thermiques, DMF, Cs_2CO_3). Pour les "mono-"et "di-amides" de LEV deux méthodes ont été comparées: l’utilisation de l’agent de couplage HATU et du système TPP / TCA. Le mélange TPP / TCA s'est avéré être plus pratique avec des réactifs disponibles et par conséquent a été appliqué pour ces séries de composés.

En ce qui concerne l'activité biologique, notre travail a démontré que l’activité native (antibactérienne) de CIP et LEV pouvait être modulée par la simple modification des substituants en C-6 et en C-7. La modification sur la position C-7 semble être plus bénéfique pour l'activité antibactérienne, notamment contre *S. aureus* pour les composés “monomères” et contre les souches résistantes de S. aureus pour les dérivés “dimères”. Contrairement à la position C-7, la substitution de la LEV en position C-6 a considérablement augmenté l'activité antiproliférative. L'influence de la fonction introduite a également été démontrée: le groupement *amide* fourni une large gamme de composés à spectre d’activité *homogène* tandis qu’un groupement *ester* a conduit à des composés à spectre d’activité *hétérogène*.

Nos travaux ont démontré le potentiel des modifications en position C-7 et C-3 des FQs et fourni la base pour de nouvelles recherches dans ce domaine.

EXPERIMENTAL PART

1. Chemistry

All reagents were of commercial quality, reagent grade and were used as supplied without further purification. LEV and CIP (>98%) were purchased from Fluka; chloromethyl alkanoates were prepared as previously described[167]. Microwave synthesis was performed in the CEM Discover® reactor at open-vessel mode. Merck silica gel 60 F_{254} plates were used for analytical TLC with UV light detection (λ = 254 nm). Preparative column chromatography purifications were performed on silica 60 Å (Merck) finer than 70 μm means of the solvent systems indicated. Melting points (mp) were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC-300 spectrometer. Data are reported in the following order: chemical shift d in ppm, signal multiplicity, value(s) of coupling constant(s), number of protons and assignment. ^{13}C NMR spectra and ^{19}F NMR spectra were recorded on a Bruker Avance-300 spectrometer. NMR spectra were recorded in deuteriochloroform unless indicated otherwise. The chemical shifts δ are expressed relative to TMS or $CFCl_3$. The attributions are reported according to the atom numbering indicated at the corresponding figures. Mass spectra were recorded on a Perkin Elmer SCIEX API 365 operating in electrospray mode. Elemental analyses were carried out by the "Service Inter-universitaire de l'ENSIACET" in Toulouse, on an EA 1110 Thermo.

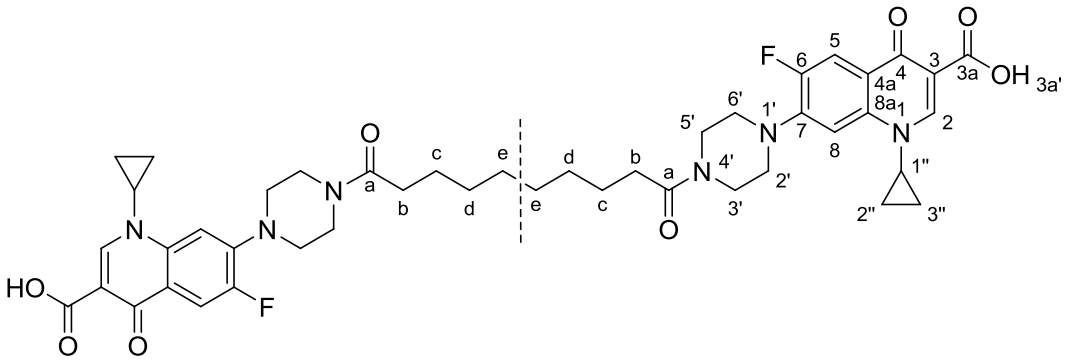


Figure 56 Atom numbering of CIP "di-amides"

General procedure for the synthesis of bis(7-(4-(alkanoyl)piperazin-1-yl)) CIP derivatives (7a-f) termed CIP "di-amides": To a solution of carboxylic diacid (0.65 mmole) in 1:1 mixture of DMF and methylenechloride (10 mL), HOBt (0.22 g, 1.63 mmole) and EDCI (0.31g, 1.63 mmole) were added and the reaction mixture was stirred at room temperature for 30 min. Then CIP (0.5 g, 1.51 mmole) was added and the mixture was stirred at r. t. for 4h. The solvent was removed under reduced pressure and the solid residue was washed with water (3 × 20 mL), mixed with 40 mL of methylenechloride. Obtained solution was filtered, filtrate was

evaporated. Crude product was purified by column chromatography on silica gel (40g, CH_2Cl_2/ MeOH, 10:0.1 – 10:1).

bis(7-(4-Decanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (7a). Purification provided the desired compound (251 mg, 48% yield) as a pale yellow solid. m.p. >260 ºC (decomp.). R_f 0.19 (CH_2Cl_2/MeOH, 10/0.75). ^{1}H NMR (500 MHz): 1.21 (dd, $^3J_{cis}$=10.5 Hz, $^3J_{trans}$=6 Hz, 4H, $C_{2''}$-H and $C_{3''}$-H), 1.35 (m, 8H, C_d-H, C_e-H), 1.40 (dd, $^3J_{cis}$=13.5 Hz, $^3J_{trans}$=6 Hz, 4H, $C_{2''}$-H and $C_{3''}$-H), 1.66 (quint, $^3J_{b-c}$=7.5 Hz, $^3J_{c-d}$=6 Hz, 4H, C_c-H), 2.38 (t, ^3J=7.5 Hz, 4H, C_b-H), 3.28 (t, ^3J=5 Hz, 4H, $C_{3'}$-H), 3.35 (t, ^3J=5 Hz, 4H, $C_{5'}$-H), 3.54 (sept, ^3J=4 and 7 Hz, 2H, $C_{1''}$-H), 3.71 (t, ^3J =5 Hz, 4H, $C_{2'}$-H), 3.87 (t, ^3J =5 Hz, 4H, $C_{6'}$-H), 7.38 (d, $^4J_{H-F}$=7 Hz, 2H, C_8-H), 8.07 (d, $^3J_{H-F}$=13 Hz, 2H, C_5-H), 8.79 (s, 2H, C_2-H), 14.89 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (125 MHz): 8.3 ($C_{2''}$, $C_{3''}$), 25.2, 29.2, 29.4, 33.2 (C_{b-e}), 35.3 ($C_{1''}$), 41.1 ($C_{3'}$), 45.4 ($C_{5'}$), 49.4 ($C_{6'}$), 50.4 ($C_{2'}$), 105.1 (d, $^3J_{C-F}$ = 2.8 Hz, C_8), 108.4 (C_3), 112.8 (d, $^2J_{C-F}$ = 23.3 Hz, C_5), 120.5 (d, $^3J_{C-F}$ = 7.9 Hz, C_{4a}), 139 (C_{8a}), 145.4 (d, $^2J_{C-F}$ = 10.5 Hz, C_7), 147.6 (C_2), 153.6 (d, $^1J_{C-F}$ = 249.6 Hz, C_6), 166.9 (C_{3a}), 171.8 (C_a), 177.1 (d, $^4J_{C-F}$ = 2.5 Hz, C_4). ^{19}F NMR (282 MHz): -121.18 – -121.25 (dd, $^3J_{H-F}$ = 12.6 Hz, $^4J_{H-F}$ = 6.9 Hz). MS CI (DCI/NH_3): $C_{44}H_{50}F_2N_6O_8$, *m/z* 829.5 $[M+H]^+$. Elemental Anal. ($C_{44}H_{50}F_2N_6O_8 \cdot H_2O$) C, H, N: calcd, 62.40, 6.19, 9.92; found, 62.41, 6.12, 9.90.

bis(7-(4-Dodecanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (7b). Purification provided the desired compound (174 mg, 31% yield) as a pale yellow solid. m.p. >260 ºC (decomp.). R_f 0.21 (CH_2Cl_2/MeOH, 10/0.75). ^{1}H NMR (500 MHz): 1.21 (dd, $^3J_{cis}$=10.5 Hz, $^3J_{trans}$=6.5 Hz, 4H, $C_{2''}$-H and $C_{3''}$-H), 1.29 – 1.36 (m, 12H, C_{d-f}-H), 1.40 (dd, $^3J_{cis}$ = 13.5 Hz, $^3J_{trans}$ = 6.5 Hz, 4H, $C_{2''}$-H and $C_{3''}$-H), 1.65 (quint, $^3J_{b-c}$ = 8 Hz, $^3J_{c-d}$ = 7 Hz, 4H, C_c-H), 2.37 (t, ^3J = 8 Hz, 4H, C_b-H), 3.28 (t, ^3J = 5 Hz, 4H, $C_{3'}$-H), 3.35 (t, ^3J = 4.5 Hz, 4H, $C_{5'}$-H), 3.54 (sept, ^3J = 4 and 7.5 Hz, 2H, $C_{1''}$-H), 3.72 (t, ^3J = 4.5 Hz, 4H, $C_{2'}$-H), 3.86 (t, ^3J = 5 Hz, 4H, $C_{6'}$-H), 7.37 (d, $^4J_{H-F}$ = 7 Hz, 2H, C_8-H), 8.03 (d, $^3J_{H-F}$ = 12.5 Hz, 2H, C_5-H), 8.77 (s, 2H, C_2-H), 14.90 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (125 MHz): 8.3 ($C_{2''}$, $C_{3''}$); 25.2, 29.4, 29.4, 33.2 (C_{b-f}); 35.3 ($C_{1''}$); 41.1 ($C_{3'}$); 45.4 ($C_{5'}$); 49.4 ($C_{6'}$); 50.3 ($C_{2'}$); 105.1 (d, $^3J_{C-F}$ = 2.8 Hz, C_8); 108.3 (C_3); 112.6 (d, $^2J_{C-F}$ = 23.1 Hz, C_5); 120.4 (d, $^3J_{C-F}$ = 7.8 Hz, C_{4a}); 138.9 (C_{8a}); 145.4 (d, $^2J_{C-F}$ = 10.4 Hz, C_7); 147.6 (C_2); 153.5 (d, $^1J_{C-F}$ = 249.7 Hz, C_6); 166.9 (C_{3a}); 171.9 (C_a), 177.1 (d, $^4J_{C-F}$ = 2.5 Hz, C_4). ^{19}F NMR (282 MHz, $CDCl_3$): -121.14 – -121.21 (dd, $^4J_{F-H}$ = 7.0 Hz, $^3J_{F-H}$ = 12.8 Hz). MS CI (DCI/NH_3): $C_{46}H_{54}F_2N_6O_8$, *m/z* 857.7 $[M+H]^+$. Elemental Anal. ($C_{48}H_{58}F_2N_6O_8 \cdot 2.8H_2O$) C, H, N: calcd, 60.90, 6.58, 9.27; found, 60.93, 6.13, 9.94.

bis(7-(4-Tetradecanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (7c). Purification provided the desired compound (400 mg, 66% yield) as a pale yellow solid. m.p. 250 ºC. R_f 0.22 (CH_2Cl_2/MeOH, 10/0.75). ^{1}H NMR (300 MHz): 1.20 (m, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.26 – 1.32 (m, 16H, $C_{d\text{-}g}$-H); 1.39 (dd, $^3J_{cis}$ = 13.8 Hz, $^3J_{trans}$ = 6.9 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.65 (quint, $^3J_{b\text{-}c}$ = 7.8 Hz, $^3J_{c\text{-}d}$ = 6.9 Hz, 4H, C_c-H); 2.37 (t, $^3J_{H\text{-}H}$ = 7.8 Hz, 4H, C_b-H); 3.29 (m, 4H, $C_{3'}$-H); 3.35 (m, 4H, $C_{5'}$-H); 3.55 (m, 2H, $C_{1''}$-H); 3.7 (t, ^3J = 4.5 Hz, 4H, $C_{2'}$-H); 3.86 (m, 4H, $C_{6'}$-H); 7.35 (d, $^4J_{H\text{-}F}$ = 6.6 Hz, 2H, C_8-H); 7.98 (d, $^3J_{H\text{-}F}$ = 12.9 Hz, 2H, C_5-H); 8.72 (s, 2H, C_2-H); 14.88 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (75 MHz): 8.2 ($C_{2''}$, $C_{3''}$); 25.2, 29.4, 29.4, 29.5, 33.2 ($C_{b\text{-}g}$); 35.3 ($C_{1''}$); 41.0 ($C_{3'}$); 45.3 ($C_{5'}$); 49.3 ($C_{6'}$); 50.3 ($C_{2'}$); 105.0 (d, $^3J_{C\text{-}F}$ = 3.0 Hz, C_8); 108.1 (C_3); 112.5 (d, $^2J_{C\text{-}F}$ = 23.2 Hz, C_5); 120.2 (d, $^3J_{C\text{-}F}$ = 7.7 Hz, C_{4a}); 138.9 (C_{8a}); 145.4 (d, $^2J_{C\text{-}F}$ = 10.2 Hz, C_7); 147.5 (C_2); 153.5 (d, $^1J_{C\text{-}F}$ = 249.7 Hz, C_6); 166.8 (C_{3a}); 171.8 (C_a), 176.9 (d, $^4J_{C\text{-}F}$ = 2.3 Hz, C_4). ^{19}F NMR (282 MHz): -121.04 – -121.11 (dd, $^4J_{F\text{-}H}$ = 7.0 Hz, $^3J_{F\text{-}H}$ = 12.9 Hz). MS CI (DCI/NH_3): $C_{48}H_{58}F_2N_6O_8$, *m/z* 885.8 $[M+H]^+$. Elemental Anal. ($C_{48}H_{58}F_2N_6O_8 \cdot H_2O$) C, H, N: calcd, 63.86, 6.65, 9.31; found, 63.90, 6.19, 10.04.

bis(7-(4-Pentadecanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (7d). Purification provided the desired compound (318 mg, 61% yield) as a pale yellow solid. m.p. 178 ºC. R_f 0.20 (CH_2Cl_2/MeOH, 10/0.75). ^{1}H NMR (300 MHz): 1.19 (m, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.24 – 1.30 (m, 18H, $C_{d\text{-}h}$-H); 1.37 (dd, $^3J_{cis}$=13.5 Hz, $^3J_{trans}$=6.9 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.63 (quint, $^3J_{b\text{-}c}$=7.5 Hz, $^3J_{c\text{-}d}$=6.9 Hz, 4H, C_c-H); 2.36 (t, $^3J_{H\text{-}H}$ = 7.5 Hz, 4H, C_b-H); 3.29 (m, 4H, $C_{3'}$); 3.36 (m, 4H, $C_{5'}$); 3.55 (m, 2H, $C_{1''}$-H); 3.71 (m, 4H, $C_{2'}$); 3.85 (m, 4H, $C_{6'}$); 7.33 (d, $^4J_{H\text{-}F}$ = 7.5 Hz, 2H, C_8-H); 7.87 (d, $^3J_{H\text{-}F}$ = 12.9 Hz, 2H, C_5-H); 8.65 (s, 2H, C_2-H); 14.87 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (75 MHz): 8.2 ($C_{2''}$, $C_{3''}$); 25.2, 29.3, 29.4, 29.5, 29.6 ($C_{c\text{-}h}$); 33.2 (C_b); 35.3 ($C_{1''}$); 41.0 ($C_{3'}$); 45.3 ($C_{5'}$); 49.3 ($C_{6'}$); 50.2 ($C_{2'}$); 105.0 (d, $^3J_{C\text{-}F}$ = 3.0 Hz, C_8); 107.9 (C_3); 112.2 (d, $^2J_{C\text{-}F}$ = 23.2 Hz, C_5); 119.8 (d, $^3J_{C\text{-}F}$ = 7.8 Hz, C_{4a}); 138.9 (C_{8a}); 145.3 (d, $^2J_{C\text{-}F}$ = 10.3 Hz, C_7); 147.3 (C_2); 153.5 (d, $^1J_{C\text{-}F}$ = 249.7 Hz, C_6); 166.7 (C_{3a}); 171.8 (C_a), 176.7 (d, $^4J_{C\text{-}F}$ = 2.5 Hz, C_4). ^{19}F NMR (282 MHz): -120.93 – -121.00 (dd, $^4J_{F\text{-}H}$ = 6.8 Hz, $^3J_{F\text{-}H}$ = 12.9 Hz). MS CI (DCI/NH_3): $C_{49}H_{60}F_2N_6O_8$, *m/z* 899.7 $[M+H]^+$. Elemental Anal. ($C_{49}H_{60}F_2N_6O_8 \cdot H_2O$) C, H, N: calcd, 64.19, 6.77, 9.17; found, 64.15, 6.76, 9.41.

bis(7-(4-Hexadecanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (7e). Purification provided the desired compound (326 mg, 55% yield) as a pale yellow solid. m.p. 144 ºC. R_f 0.20 (CH_2Cl_2/MeOH, 10/0.75). ^{1}H NMR (300 MHz): 1.20 (m, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.24 – 1.32 (m, 20H, $C_{d\text{-}h}$-H); 1.39 (dd, $^3J_{cis}$=13.5 Hz, $^3J_{trans}$=6.9 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.65 (quint, $^3J_{b\text{-}c}$=7.5 Hz, $^3J_{c\text{-}d}$=7.0 Hz, 4H, C_c-H); 2.37 (t, $^3J_{H\text{-}H}$

= 7.5 Hz, 4H, C_b-H); 3.29 (m, 4H, $C_{3'}$-H); 3.36 (m, 4H, $C_{5'}$-H); 3.55 (m, 2H, $C_{1''}$-H); 3.72 (m, 4H, $C_{2'}$-H); 3.86 (m, 4H, $C_{6'}$-H); 7.35 (d, $^4J_{H\text{-}F}$ = 7.2 Hz, 2H, C_8-H); 7.94 (d, $^3J_{H\text{-}F}$ = 12.9 Hz, 2H, C_5-H); 8.69 (s, 2H, C_2-H); 14.88 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (75 MHz): 8.3 ($C_{2''}$, $C_{3''}$); 25.2, 29.4, 29.4, 29.4, 29.6, 29.6 ($C_{c\text{-}h}$); 33.2 (C_b); 35.4 ($C_{1''}$); 41.1 ($C_{3'}$); 45.4 ($C_{5'}$); 49.4 ($C_{6'}$); 50.3 ($C_{2'}$); 105.1 (d, $^3J_{C\text{-}F}$ = 3.0 Hz, C_8); 107.9 (C_3); 112.4 (d, $^2J_{C\text{-}F}$ = 23.2 Hz, C_5); 120.1 (d, $^3J_{C\text{-}F}$ = 7.8 Hz, C_{4a}); 139.0 (C_{8a}); 145.4 (d, $^2J_{C\text{-}F}$ = 10.3 Hz, C_7); 147.5 (C_2); 153.5 (d, $^1J_{C\text{-}F}$ = 249.8 Hz, C_6); 166.8 (C_{3a}); 171.9 (C_a), 176.9 (d, $^4J_{C\text{-}F}$ = 2.6 Hz, C_4). ^{19}F NMR (282 MHz): -121.00 – -121.07 (dd, $^4J_{F\text{-}H}$ = 7.0 Hz, $^3J_{F\text{-}H}$ = 12.7 Hz). MS CI (DCI/NH_3): $C_{50}H_{62}F_2N_6O_8$, *m/z* 913.7 $[M+H]^+$. Elemental Anal. ($C_{50}H_{62}F_2N_6O_8 \cdot 2H_2O$) C, H, N: calcd, 63.29, 6.96, 8.86; found, 63.28, 6.85, 9.22.

bis(7-(4-Octadecanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (7f). Purification provided the desired compound (325 mg, 53% yield) as a pale yellow solid. m.p. 138 ºC. R_f 0.22 (CH_2Cl_2/MeOH, 10/0.75). ^{1}H NMR (300 MHz): 1.20 (m, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.23 – 1.32 (m, 24H, $C_{d\text{-}i}$-H); 1.39 (dd, $^3J_{cis}$=13.5 Hz, $^3J_{trans}$=6.9 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.64 (quint, $^3J_{b\text{-}c}$=7.5 Hz, $^3J_{c\text{-}d}$=6.6 Hz, 4H, C_c-H); 2.37 (t, $^3J_{H\text{-}H}$ = 7.5 Hz, 4H, C_b-H); 3.29 (m, 4H, $C_{3'}$-H); 3.36 (m, 4H, $C_{5'}$-H); 3.55 (m, 2H, $C_{1''}$-H); 3.72 (m, 4H, $C_{2'}$-H); 3.86 (m, 4H, $C_{6'}$-H); 7.33 (d, $^4J_{H\text{-}F}$ = 6.9 Hz, 2H, C_8-H); 7.89 (d, $^3J_{H\text{-}F}$ = 12.9 Hz, 2H, C_5-H); 8.66 (s, 2H, C_2-H); 14.88 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (75 MHz): 8.2 ($C_{2''}$, $C_{3''}$); 25.2, 29.4, 29.4, 29.4, 29.5, 29.6 ($C_{c\text{-}i}$); 33.2 (C_b); 35.3 ($C_{1''}$); 41.0 ($C_{3'}$); 45.3 ($C_{5'}$); 49.3 ($C_{6'}$); 50.1 ($C_{2'}$); 105.0 (d, $^3J_{C\text{-}F}$ = 3.0 Hz, C_8); 107.9 (C_3); 112.2 (d, $^2J_{C\text{-}F}$ = 23.2 Hz, C_5); 119.9 (d, $^3J_{C\text{-}F}$ = 7.8 Hz, C_{4a}); 138.9 (C_{8a}); 145.3 (d, $^2J_{C\text{-}F}$ = 10.3 Hz, C_7); 147.4 (C_2); 153.5 (d, $^1J_{C\text{-}F}$ = 249.8 Hz, C_6); 166.7 (C_{3a}); 171.9 (C_a); 176.8 (d, $^4J_{C\text{-}F}$ = 2.6 Hz, C_4). ^{19}F NMR (282 MHz): -120.94 – -121.01 ($^4J_{F\text{-}H}$ = 7.0 Hz, $^3J_{F\text{-}H}$ = 12.7 Hz). MS CI (DCI/NH_3): $C_{52}H_{66}F_2N_6O_8$, *m/z* 941.8 $[M+H]^+$. Elemental Anal. ($C_{52}H_{66}F_2N_6O_8 \cdot 2H_2O$) C, H, N: calcd, 63.93, 7.17, 8.61; found, 63.94, 7.01, 9.22.

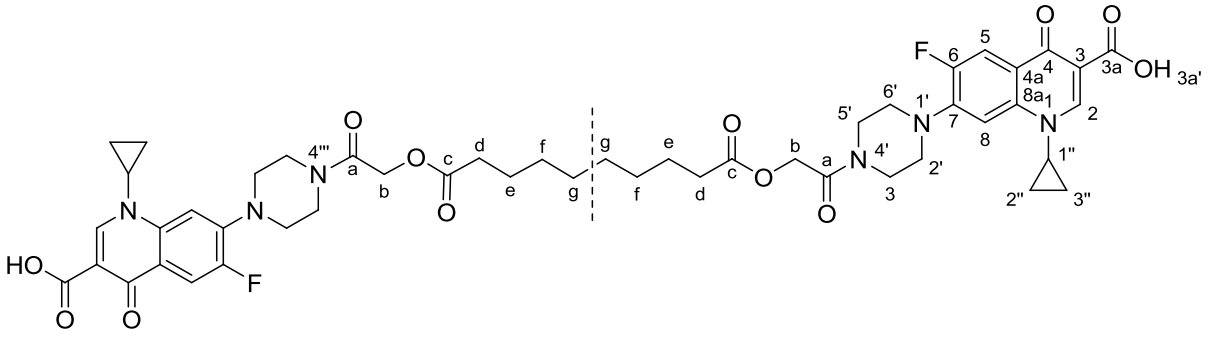


Figure 57 Atom numbering of CIP "di-oxoethyl esters"

General procedure for the synthesis of bis(7-(4-(2-Oxoethylalcanoate)piperazin-1-yl)) CIP derivatives (9a-f) termed CIP "di-oxoethyl esters". To a solution of carboxylic diacid (0.5 mmole) in DMF (10 mL), Cs_2CO_3 (0.32 g, 1.00 mmole) was added and the reaction mixture was stirred at 50-60ºC for 1 hour. Then *N*-chloroacetylciprofloxacin (0.42 g, 1.03 mmole) and TBAI (0.37 g, 1.00 mmole) were added and the mixture was stirred at the previously mentioned temperature for 72h. The solvent was removed under reduced pressure and 20 mL of aqueous HCl 1M was added to the residue. The obtained solid was filtered and washed with aqueous HCl 1M, water, ethanol and petroleum ether. The remaining solid residue was purified by column chromatography (CH_2Cl_2/MeOH from 10/0.3 to 10/0.5).

bis(7-(4-Oxoethyldecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (9a). Obtained from decanedioïc acid (n=8) as a pale yellow solid in 42 % yield (200 mg). Due to the low solubility of this compound, even in DMSO-d_6, no ^{13}C NMR could be obtained. ^{1}H NMR (300 MHz, DMSO-d_6): 1.21-1.34 (m, 12H, $C_{2''}$-H, $C_{3''}$-H, C_f-H, C_g-H); 1.40 (dd, $^3J_{cis}$=13.5 Hz, $^3J_{trans}$=6.9 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.67 (quint, $^3J_{d-e}$=7.5 Hz, $^3J_{e-f}$=7.0, 4H, C_e-H); 2.45 (t, $^3J_{H-H}$ = 7.5Hz, 4H, C_d-H); 3.32 – 3.37 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.54 (m, 2H, $C_{1''}$-H); 3.66 (m, 4H, $C_{2'}$-H); 3.87 (m, 4H, $C_{6'}$-H); 4.78 (s, 4H, C_b-H); 7.38 (d, $^4J_{H-F}$ = 6.6 Hz, 2H, C_8-H); 8.07 (d, $^3J_{H-F}$ = 12.6Hz, 2H, C_5-H); 8.79 (s, 2H, C_2-H); 14.86 (br s, 2H, $O_{3a'}$-H). ^{19}F NMR (282 MHz, DMSO-d_6): -121.28 – -121.35 (dd, $^3J_{H-F}$ = 12.7 Hz, $^4J_{H-F}$ = 7.0 Hz). MS CI (DCI/NH_3): $C_{48}H_{54}F_2N_6O_{12}$, *m/z* 945.6 $[M+H]^+$. Elemental Anal. ($C_{48}H_{54}F_2N_6O_{12} \cdot 1.2\ H_2O$); C, H, N: calcd, 59.68, 5.88, 8.70; found, 59.65, 5.96, 8.67.

bis(7-(4-Oxoethyldodecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (9b). Obtained from dodecanedioïc acid (n=10) as a pale yellow solid in 47 % yield (200 mg). ^{1}H NMR (300 MHz, DMSO-d_6): 1.21 – 1.32 (m, 16H, $C_{2''}$-H, $C_{3''}$-H, C_{f-h}-H); 1.40 (dd, $^3J_{cis}$=13.2 Hz, $^3J_{trans}$=6.6 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.70 (quint, $^3J_{d-e}$=7.5 Hz, $^3J_{e-f}$=7.0 Hz, 4H, C_e-H); 2.45 (t, $^3J_{H-H}$ = 7.5 Hz, 4H, C_d-H); 3.32 – 3.37 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.55 (m, 2H, $C_{1''}$-H); 3.66 (m, 4H, $C_{2'}$-H); 3.88 (m, 4H, $C_{6'}$-H); 4.78 (s, 4H, C_b-H); 7.38 (d, $^4J_{H-F}$ = 6.5 Hz, 2H, C_8-H); 8.07 (d, $^3J_{H-F}$ = 12.6 Hz, 2H, C_5-H); 8.78 (s, 2H, C_2-H); 14.89 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (125 MHz, DMSO-d_6): 8.0 ($C_{2''}$, $C_{3''}$); 24.9 (C_h); 29.2, 29.1, 28.8 (C_{e-g}); 33.7 (C_d); 36.3 ($C_{1''}$); 41.5 ($C_{3'}$); 44.5 ($C_{5'}$); 48.9 ($C_{2'}$); 49.2 ($C_{6'}$); 61.6 (C_b); 107.1 (d, $^3J_{C-F}$ = 3 Hz, C_8); 107.4 (C_3); 111.5 (d, $^2J_{C-F}$ = 24 Hz, C_5); 119.7 (d, $^3J_{C-F}$ = 8 Hz, C_{4a}); 139.6 (C_{8a}); 145.3 (d, $^2J_{C-F}$ = 10.4 Hz, C_7); 148.5 (C_2); 153.4 (d, $^1J_{C-F}$ = 249.0 Hz, C_6); 165.5 (C_c); 166.3 (C_{3a}); 172.9 (C_a); 176.8 (d, $^4J_{C-F}$ = 2.3 Hz, C_4). ^{19}F NMR (282 MHz, DMSO-d_6): -121.31 – -121.38 (dd,

$^{3}J_{H\text{-}F}$=12.5 Hz, $^{4}J_{H\text{-}F}$=6.6Hz). MS CI (DCI/NH_3): $C_{50}H_{58}F_2N_6O_{12}$, m/z found, 973.6 $[M+H]^+$ Elemental Anal. ($C_{50}H_{58}F_2N_6O_{12}$ · 1.9 H_2O); C, H, N: calcd, 59.65, 6.18, 8.35; found, 59.49, 5.94, 8.56.

bis(7-(4-Oxoethyltetradecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (9c). Obtained from tetradecanedioïc acid (n=12) as a pale yellow solid in 56% yield (250 mg). ^{1}H NMR (500 MHz): 1.20 – 1.32 (m, 20H, $C_{2''}$-H, $C_{3''}$-H, $C_{f\text{-}i}$-H); 1.43 (dd, $^{3}J_{cis}$ = 13.2 Hz, $^{3}J_{trans}$ = 6.6 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.69 (quint, $^{3}J_{d\text{-}e}$=7.5 Hz, $^{3}J_{e\text{-}f}$ = 6.9 Hz, 4H, C_e-H); 2.45 (t, $^{3}J_{H\text{-}H}$ = 7.5 Hz, 4H, C_d-H); 3.32 – 3.37 (2m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.55 (m, 2H, $C_{1''}$-H); 3.66 (m, 4H, $C_{2'}$-H); 3.87 (m, 4H, $C_{6'}$-H); 4.78 (s, 4H, C_b-H); 7.38 (d, $^{4}J_{H\text{-}F}$ = 6.6 Hz, 2H, C_8-H); 8.05 (d, $^{3}J_{H\text{-}F}$ = 12.6 Hz, 2H, C_5-H); 8.79 (s, 2H, C_2-H); 14.87 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (125 MHz): 8.2 ($C_{2''}$, $C_{3''}$); 24.9 (C_i); 29.5, 29.5, 29.4, 29.3 ($C_{e\text{-}h}$); 33.9 (C_d); 35.3 ($C_{1''}$); 41.5 ($C_{3'}$); 44.5 ($C_{5'}$); 49.3 ($C_{2'}$); 50.0 ($C_{6'}$); 58.6 (C_b); 105.3 (d, $^{3}J_{C\text{-}F}$ = 2.9 Hz, C_8); 108.2 (C_3); 112.7 (d, $^{2}J_{C\text{-}F}$ = 23.3 Hz, C_5); 120.5 (d, $^{3}J_{C\text{-}F}$ = 7.9 Hz, C_{4a}); 138.9 (C_{8a}); 145.2 (d, $^{2}J_{C\text{-}F}$ = 10.5 Hz, C_7); 147.6 (C_2); 153.5 (d, $^{1}J_{C\text{-}F}$ = 251.4 Hz, C_6); 165.3 (C_c); 166.8 (C_{3a}); 173.5 (C_a); 177.1 (d, $^{4}J_{C\text{-}F}$ = 2.5 Hz, C_4). ^{19}F NMR (282 MHz): 121.28 – -121.35 (dd, $^{3}J_{H\text{-}F}$ = = 12.9 Hz, $^{4}J_{H\text{-}F}$ = 7.0 Hz). MS CI (DCI/NH_3): $C_{52}H_{62}F_2N_6O_{12}$, *m/z* 1001.7 $[M+H]^+$. Elemental Anal. ($C_{52}H_{62}F_2N_6O_{12}$ · 0.9 H_2O); C, H, N: calcd, 61.39, 6.32, 8.26; found, 61.22, 6.24, 8.66.

bis(7-(4-Oxoethylpentadecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (9d). Obtained from pentadecanedioïc acid (n=13) as a pale yellow solid in 56% yield (260 mg). ^{1}H NMR (300 MHz): 1.21 – 1.45 (m, 26H, $C_{2''}$-H, $C_{3''}$-H, $C_{f\text{-}j}$-H,); 1.65 (quint, $^{3}J_{d\text{-}e}$ = 7.8 Hz, $^{3}J_{e\text{-}f}$ = 7.2 Hz, 4H, C_e-H); 2.45 (t, $^{3}J_{H\text{-}H}$ = 7.8 Hz, 4H, C_d-H); 3.32 – 3.38 (2m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.56 (m, 2H, $C_{1''}$-H); 3.66 (m, 4H, $C_{2'}$-H); 3.87 (m, 4H, $C_{6'}$-H); 4.78 (s, 4H, C_b-H); 7.37 (d, $^{4}J_{H\text{-}F}$ = 6.9 Hz, 2H, C_8-H); 8.00 (d, $^{3}J_{H\text{-}F}$ = 12.6 Hz, 2H, C_5-H); 8.74 (s, 2H, C_2-H); 14.87 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (75 MHz): 8.2 ($C_{2''}$, $C_{3''}$); 24.7 (C_j); 29.0, 29.2, 29.3, 29.4, 29.5 ($C_{e\text{-}i}$); 33.9 (C_d); 35.3 ($C_{1''}$); 41.5 ($C_{3'}$); 44.2 ($C_{5'}$); 49.2 ($C_{2'}$); 49.8 ($C_{6'}$); 60.9 (C_b); 105.2 (d, $^{3}J_{C\text{-}F}$ = 2.7 Hz, C_8); 107.9 (C_3); 112.3 (d, $^{2}J_{C\text{-}F}$ = 23.1 Hz, C_5); 120.1 (d, $^{3}J_{C\text{-}F}$ = 7.7 Hz, C_{4a}); 138.9 (C_{8a}); 145.2 (d, $^{2}J_{C\text{-}F}$ = 10.4 Hz, C_7); 147.4 (C_2); 153.5 (d, $^{1}J_{C\text{-}F}$ = 249.8 Hz, C_6); 165.2 (C_c); 166.6 (C_{3a}); 173.4 (C_a); 176.8 (d, $^{4}J_{C\text{-}F}$ = 2.4 Hz, C_4). ^{19}F NMR (282 MHz): -121.06 – -121.13 (dd, $^{3}J_{H\text{-}F}$ = 12.7 Hz, $^{4}J_{H\text{-}F}$ = 6.0 Hz); MS CI (DCI/NH_3): $C_{53}H_{64}F_2N_6O_{12}$, *m/z* 1015.4 $[M+H]^+$. Elemental Anal. ($C_{53}H_{64}F_2N_6O_{12}$ · 1H_2O) C, H, N: calcd, 61.62, 6.44, 8.13; found, 61.78, 6.56, 8.12.

bis(7-(4-Oxoethylhexadecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (9e). Obtained from hexadecanedioïc acid (n=14) as a pale yellow solid in 41% yield (190 mg). ^{1}H NMR (500 MHz) : 1.21 (dd, $^{3}J_{cis}$ = 10.5 Hz, $^{3}J_{trans}$ = 7.5 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.24 –1.34 (m, 20H, $C_{f\text{-}j}$-H); 1.40 (dd, $^{3}J_{cis}$ = 14.0 Hz, $^{3}J_{trans}$ = 7.5 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.67 (quint, $^{3}J_{d\text{-}e}$ = 7.5 Hz, $^{3}J_{e\text{-}f}$ = 7.5 Hz, 4H, C_e-H); 2.45 (t, $^{3}J_{H\text{-}H}$ = 7.5 Hz, 4H, C_d-H); 3.31 – 3.38 (2 m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.55 (quint, ^{3}J = 3.5 and 7.5 Hz, 2H, $C_{1''}$-H); 3.66 (m, 4H, $C_{2'}$-H); 3.87 (m, 4H, $C_{6'}$-H); 4.78 (s, 4H, C_b-H); 7.37 (d, $^{4}J_{H\text{-}F}$ = 7.0 Hz, 2H, C_8-H); 8.00 (d, 2H, $^{3}J_{H\text{-}F}$ = 12.5 Hz, C_5-H); 8.74 (s, 2H, C_2-H); 14.87 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (125 MHz): 8.3 ($C_{2''}$, $C_{3''}$); 24.8 (C_j); 29.6, 29.5, 29.4, 29.2, 29.0 ($C_{e\text{-}i}$); 33.9 (C_d); 35.3 ($C_{1''}$); 41.0 ($C_{3'}$); 45.3 ($C_{5'}$); 49.2 ($C_{2'}$); 49.9 ($C_{6'}$); 60.9 (C_b); 105.2 (d, $^{3}J_{C\text{-}F}$ = 3.0 Hz, C_8); 108.2 (C_3); 112.6 (d, $^{2}J_{C\text{-}F}$ = 23.1 Hz, C_5); 120.4 (d, $^{3}J_{C\text{-}F}$ = 7.8 Hz, C_{4a}); 138.9 (C_{8a}); 145.2 (d, $^{2}J_{C\text{-}F}$ = 10.3 Hz, C_7); 147.6 (C_2); 153.5 (d, $^{1}J_{C\text{-}F}$ = 249.8 Hz, C_6); 165.3 (C_c); 166.8 (C_{3a}); 173.5 (C_a); 177.0 (d, $^{4}J_{C\text{-}F}$ = 2.5 Hz, C_4). ^{19}F NMR (282 MHz): -121.20 – -121.27 (dd, $^{3}J_{H\text{-}F}$ = 12.7 Hz, $^{4}J_{H\text{-}F}$ = 7.0 Hz); MS CI (DCI/NH_3): $C_{54}H_{66}F_2N_6O_{12}$, *m/z* 1029.6 $[M+H]^+$. Elemental Anal. ($C_{54}H_{66}F_2N_6O_{12} \cdot 2.6H_2O$) C, H, N: calcd, 60.28, 6.67, 7.81; found, 60.20, 6.50, 7.51.

bis(7-(4-Oxoethyloctadecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid) (9f). Obtained from octadecanedioïc acid (n=16) as a pale yellow solid in 75% yield (330 mg). ^{1}H NMR (500 MHz) : 1.21 (dd, $^{3}J_{cis}$ = 10.5 Hz, $^{3}J_{trans}$ = 7.0 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H); 1.24 – 1.34 (m, 24H, $C_{f\text{-}k}$-H), 1.40 (dd, $^{3}J_{cis}$ = 13.5 Hz, $^{3}J_{trans}$ = 7.0 Hz, 4H, $C_{2''}$-H, $C_{3''}$-H), 1.68 (quint, $^{3}J_{d\text{-}e}$ = 7.5 Hz, $^{3}J_{e\text{-}f}$ = 7.0 Hz, 4H, C_e-H); 2.45 (t, $^{3}J_{H\text{-}H}$ = 7.5 Hz, 4H, C_d-H); 3.31 – 3.38 (2 m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.55 (quint, ^{3}J = 3.5 and 7.0 Hz, 2H, $C_{1''}$-H); 3.66 (m, 4H, $C_{2'}$-H); 3.87 (m, 4H, $C_{6'}$-H); 4.78 (s, 4H, C_b-H); 7.37 (d, $^{4}J_{H\text{-}F}$ = 7.0 Hz, 2H, C_8-H); 8.05 (d, $^{3}J_{H\text{-}F}$ = 12.5 Hz, 2H, C_5-H); 8.78 (s, 2H, C_2-H); 14.87 (br s, 2H, $O_{3a'}$-H). ^{13}C NMR (125 MHz): 8.3 ($C_{2''}$, $C_{3''}$); 24.8 (C_k); 29.1, 29.2, 29.4, 29.5, 29.6, 29.7 ($C_{e\text{-}j}$); 33.9 (C_d); 35.3 ($C_{1''}$); 41.5 ($C_{3'}$); 44.5 ($C_{5'}$); 49.3 ($C_{2'}$); 50.0 ($C_{6'}$); 60.9 (C_b); 105.2 (d, $^{3}J_{C\text{-}F}$ = 2.9 Hz, C_8); 108.3 (C_3); 112.7 (d, $^{2}J_{C\text{-}F}$ = 23.1 Hz, C_5); 120.6 (d, $^{3}J_{C\text{-}F}$ = 7.8 Hz, C_{4a}); 138.9 (C_{8a}); 145.2 (d, $^{2}J_{C\text{-}F}$ = 10.5 Hz, C_7); 147.7 (C_2); 153.6 (d, $^{1}J_{C\text{-}F}$ = 249.8 Hz, C_6); 165.3 (C_c); 166.8 (C_{3a}); 173.5 (C_a); 177.1 (d, $^{4}J_{C\text{-}F}$ = 2.4 Hz, C_4). ^{19}F NMR (282 MHz): -121.23 – -121.30 (dd, $^{3}J_{H\text{-}F}$ = 12.7 Hz, $^{4}J_{H\text{-}F}$ = 7.0 Hz); MS CI (DCI/NH_3): $C_{56}H_{70}F_2N_6O_{12}$, *m/z* 1057.6 $[M+H]^+$. Elemental Anal. ($C_{56}H_{70}F_2N_6O_{12} \cdot 1H_2O$) C, H, N: calcd, 62.56, 6.75, 7.82; found, 62.29, 6.78, 7.92.

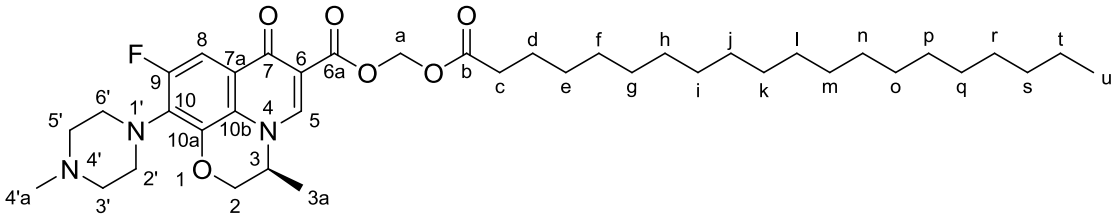


Figure 58 Atom numbering for LEV "mono-acyloxymethyl esters"

General procedure for the synthesis of (3S)-6-[(Alkanoyloxy)methyl]9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylates termed LEV "acyloxymethyl esters" (14a-i) under thermal conditions (Δ): LEV (180 mg, 0.5 mmol) and grinded K_2CO_3 (69 mg, 0.5 mmol) were mixed with 20 mL of dry DMF and left stirring at 90ºC for 20 min. The corresponding chloromethyl alkanoate was added (0.5 mmol) and reaction mixture was stirred at 90ºC for 1h. Then it was diluted with dichloromethane to 50 mL, washed by 5% aqueous solution of sodium chloride (5×100 mL). Organic layer was dried over anhydrous $MgSO_4$ and concentrated on rotary evaporator to provide crude product which was further purified by column chromatography.

Procedure for the synthesis of 14a under microwave activation (MW): A mixture of LEV (100 mg, 0.276 mmol) and K_2CO_3 (76 mg, 0.552 mmol) in acetonitrile (2.5 mL) was heated to 300 W/ 90 ºC and maintained at this temperature for 20 min. After cooling, (*n*-Bu)$_4$NI (20 mg, 0.055 mmol) and chloromethylpropanoate (52 μL, 0.414 mmol) were added. The reaction mixture was vigorously stirred at 300 W/90 ºC for 1 h. The mixture was cooled and concentrated in vacuo. Water (50 mL) and CH_2Cl_2 (50 mL) were then added to the residue. The organic layer was washed with water (2 × 50 mL). This aqueous layer was extracted with CH_2Cl_2 (30 mL). The organic layers were separated, washed with water (3 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated. The remaining residue was purified by flash chromatography on silica gel.

(3S)-6-[(Butanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14a). The procedure described above (Δ) applied to $C_3H_7COOCH_2Cl$ (62 μL, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 35-70μm; NEt_3/Acetone 2/100) and subsequent acetone wash (10 mL) 143 mg (59%) of the title compound as a white solid. R_f 0.19 (CH_2Cl_2/MeOH, 90/10). m.p. 210ºC. ^{1}H NMR (300 MHz): 0.96 (t, $^3J_{H\text{-}H}$ = 7.2 Hz, 3H, C_e-H); 1.52 (d, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_{3a}-H); 1.68 (sext, $^3J_{H\text{-}H}$ = 7.4 Hz, 2H, C_d-H); 2.37 (s, 3H, $C_{4'a}$-H); 2.39 (t, $^3J_{H\text{-}H}$ = 7.4 Hz, 2H, C_c-H);

2.55 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.35 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.28 – 4.61 (m, 3H, C_2-H, C_3-H); 5.95 (AB system, $^2J_{H\text{-}H}$ = 5.6 Hz, 2H, C_a-H); 7.38 (d, $^3J_{H\text{-}F}$ = 12.5 Hz, 1H, C_8-H); 8.17 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 13.5 (C_e); 18.1 (C_d); 18.3 (C_{3a}); 35.8 (C_c); 46.4 ($C_{4'a}$); 50.5 (d, $^4J_{C\text{-}F}$ = 4.2 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.2 (d, $^2J_{C\text{-}F}$ = 24.1 Hz, C_8); 107.9 (C_6); 122.9 (d, $^3J_{C\text{-}F}$ = 8.5 Hz, C_{7a}); 123.5 (d, $^4J_{C\text{-}F}$ = 1.4 Hz, C_{10b}); 131.9 (d, $^2J_{C\text{-}F}$ = 14.4 Hz, C_{10}); 139.6 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C\text{-}F}$ = 2.7 Hz, C_7); 172.6 (C_b). ^{19}F NMR (282 MHz): -120.9 (d, $^3J_{F\text{-}H}$ = 12.4 Hz). MS-ESI (positive mode, MeOH) *m/z* 462.5 [M+H$^+$]. Elemental Anal. ($C_{23}H_{28}FN_3O_6$); C, H, N: calcd, 59.86, 6.12, 9.11; found, 59.57, 5.88, 9.02.

(3S)-6-[(Hexanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14b). The procedure described above (Δ) applied to $C_5H_{11}COOCH_2Cl$ (82 mg, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 35-70µm; NEt_3/Acetone 1/100) 171 mg (69%) of the title compound as a white solid. R_f 0.22 (CH_2Cl_2/MeOH, 90/10). m.p. 190ºC. ^{1}H NMR (300 MHz): 0.89 (t, $^3J_{H\text{-}H}$ = 6.9 Hz, 3H, C_g-H); 1.22 – 1.37 (m, 4H, C_e-H, C_f-H); 1.53 (d, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_{3a}-H); 1.66 (m, 2H, C_d-H); 2.38 (s, 3H, $C_{4'a}$-H); 2.40 (t, $^3J_{H\text{-}H}$ = 7.4 Hz, 2H, C_c-H); 2.57 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.36 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.32 – 4.55 (m, 3H, C_2-H, C_3-H); 5.95 (AB system, $^2J_{H\text{-}H}$ = 5.6 Hz, 2H, C_a-H); 7.44 (d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.21 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 13.9 (C_g); 18.3 (C_{3a}); 22.3 (C_f); 24.3 (C_e); 31.2 (C_d); 34.0 (C_c); 46.4 ($C_{4'a}$); 50.5 (d, $^4J_{C\text{-}F}$ = 4.2 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.4 (d, $^2J_{C\text{-}F}$ = 23.7 Hz, C_8); 108.0 (C_6); 123.0 (d, $^3J_{C\text{-}F}$ = 7.8 Hz, C_{7a}); 123.5 (d, $^4J_{C\text{-}F}$ = 1.4 Hz, C_{10b}); 131.9 (d, $^2J_{C\text{-}F}$ = 14.2 Hz, C_{10}); 139.6 (d, $^3J_{C\text{-}F}$ = 6.7 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C\text{-}F}$ = 2.3 Hz, C_7); 172.8 (C_b). ^{19}F NMR (282 MHz): -121.0 (d, $^3J_{F\text{-}H}$ = 11.4 Hz). MS-ESI (positive mode, MeOH) *m/z* 490.8 [M+H$^+$]. Elemental Anal. ($C_{25}H_{32}FN_3O_6$); C, H, N: calcd, 61.34, 6.59, 8.58; found, 61.52, 6.48, 8.49.

(3S)-6-[(Octanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14c). The procedure described above (Δ) applied to $C_7H_{15}COOCH_2Cl$ (96 mg, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 35-70µm; NEt_3/Acetone 1/100) 192 mg (75%) of the title compound as a white solid. R_f 0.23 (CH_2Cl_2/MeOH, 90/10). m.p. 189ºC. ^{1}H NMR (300 MHz): 0.86 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_i-H); 1.21 – 1.36 (m, 8H, $C_{e\text{-}h}$-H);1.53 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.60 – 1.70 (m, 2H, C_d-H); 2.38 (s, 3H, $C_{4'a}$-H); 2.40 (t, $^3J_{H\text{-}H}$ = 7.2 Hz, 2H, C_c-H); 2.56 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.36 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.33 – 4.54 (m, 3H, C_2-H, C_3-H); 5.96 (AB system, $^2J_{H\text{-}H}$ = 5.7

Hz, 2H, C_a-H); 7.41 (d, $^3J_{H-F}$ = 12.0 Hz, 1H, C_8-H); 8.19 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 14.0 (C_i); 18.3 (C_{3a}); 22.6 (C_h); 24.6 (C_g); 28.9, 29.0 (C_f, C_e); 31.6 (C_d); 34.0 (C_c); 46.4 ($C_{4'a}$); 50.5 (d, $^4J_{C-F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.3 (d, $^2J_{C-F}$ = 24.0 Hz, C_8); 107.9 (C_6); 123.0 (d, $^3J_{C-F}$ = 8.5 Hz, C_{7a}); 123.5 (d, $^4J_{C-F}$ = 1.4 Hz, C_{10b}); 131.9 (d, $^2J_{C-F}$ = 14.2 Hz, C_{10}); 139.6 (d, $^3J_{C-F}$ = 6.8 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C-F}$ = 245.9 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C-F}$ = 2.6 Hz, C_7); 172.8 (C_b). ^{19}F NMR (282 MHz): -120.9 (d, $^3J_{F-H}$ = 11.3 Hz). MS-ESI (positive mode, MeOH) *m/z* 518.8 $[M+H]^+$. Elemental Anal. ($C_{27}H_{36}FN_3O_6$); C, H, N: calcd, 62.65, 7.01, 8.12; found, 62.67, 6.86, 8.12.

(3S)-6-[(Decanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14d). The procedure described above (Δ) applied to $C_9H_{19}COOCH_2Cl$ (110 mg, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 35-70µm; NEt_3/Acetone 1/100) 215 mg (79%) of the title compound as a white solid. R_f 0.24 (CH_2Cl_2/MeOH, 90/10). m.p. 189ºC. ^{1}H NMR (300 MHz): 0.86 (t, $^3J_{H-H}$ = 6.8 Hz, 3H, C_k-H); 1.19 – 1.36 (m, 12H, C_{e-j}-H);1.53 (d, $^3J_{H-H}$ = 6.9 Hz, 3H, C_{3a}-H); 1.64 (m, 2H, C_d-H); 2.38 (s, 3H, $C_{4'a}$-H); 2.40 (t, $^3J_{H-H}$ = 7.5 Hz, 2H, C_c-H); 2.57 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.36 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.32 – 4.55 (m, 3H, C_2-H, C_3-H); 5.95 (AB system, $^2J_{H-H}$ = 5.7 Hz, 2H, C_a-H); 7.41 (d, $^3J_{H-F}$ = 12.6 Hz, 1H, C_8-H); 8.20 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 14.1 (C_k); 18.3 (C_{3a}); 22.6 (C_j); 24.6 (C_i); 29.0, 29.2, 29.2, 29.4 (C_{e-h}); 31.8 (C_d); 34.0 (C_c); 46.4 ($C_{4'a}$); 50.5 (d, $^4J_{C-F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.3 (d, $^2J_{C-F}$ = 24.1 Hz, C_8); 107.9 (C_6); 123.0 (d, $^3J_{C-F}$ = 8.5 Hz, C_{7a}); 123.5 (d, $^4J_{C-F}$ = 1.4 Hz, C_{10b}); 131.9 (d, $^2J_{C-F}$ = 14.2 Hz, C_{10}); 139.6 (d, $^3J_{C-F}$ = 6.8 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C-F}$ = 245.9 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C-F}$ = 2.7 Hz, C_7); 172.8 (C_b). ^{19}F NMR (282 MHz): -121.0 (d, $^3J_{F-H}$ = 11.3 Hz). MS-ESI (positive mode, MeOH) *m/z* 546.5 $[M+H]^+$. Elemental Anal. ($C_{29}H_{40}FN_3O_6 \cdot 1.2H_2O$); C, H, N: calcd, 61.40, 7.53, 7.41; found, 61.33, 7.00, 7.39.

(3S)-6-[(Dodecanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14e). The procedure described above (Δ) applied to $C_{11}H_{23}COOCH_2Cl$ (124 mg, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 35-70µm; NEt_3/Acetone 1/100) 233 mg (79%) of the title compound as a white solid. R_f 0.25 (CH_2Cl_2/MeOH, 99/1). m.p. 189ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^3J_{H-H}$ = 6.6 Hz, 3H, C_m-H); 1.21 – 1.35 (m, 16H, C_{e-l}-H); 1.53 (d, $^3J_{H-H}$ = 6.9 Hz, 3H, C_{3a}-H); 1.65 (m, 2H, C_d-H); 2.37 (s, 3H, $C_{4'a}$-H); 2.40 (t, $^3J_{H-H}$ = 7.5 Hz, 2H, C_c-H); 2.56 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.36 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.32 – 4.54 (m, 3H, C_2-H, C_3-H); 5.96 (AB system, $^2J_{H-H}$ = 5.4 Hz, 2H, C_a-H); 7.44 (d, $^3J_{H-F}$ = 12.6 Hz, 1H, C_8-H); 8.20 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 14.1

(C_m); 18.3 (C_{3a}); 22.7 (C_l); 24.6 (C_k); 29.1, 29.3, 29.3, 29.5, 29.6 ($C_{e\text{-}j}$); 31.9 (C_d); 34.0 (C_c); 46.4 ($C_{4'a}$); 50.6 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.4 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C_8); 108.0 (C_6); 123.0 (d, $^3J_{C\text{-}F}$ = 8.5 Hz, C_{7a}); 123.5 (d, $^4J_{C\text{-}F}$ = 1.4 Hz, C_{10b}); 131.9 (d, $^2J_{C\text{-}F}$ = 14.2 Hz, C_{10}); 139.6 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C\text{-}F}$ = 2.6 Hz, C_7); 172.8 (C_b). ^{19}F NMR (282 MHz): -120.9 (d, $^3J_{F\text{-}H}$ = 11.3 Hz). MS-ESI (positive mode, MeOH) *m/z* 574.7 $[M+H]^+$. Elemental Anal. ($C_{31}H_{44}FN_3O_6 \cdot 0.4H_2O$); C, H, N: calcd, 64.10, 7.77, 7.23; found, 64.01, 7.52, 7.20.

(3S)-6-[(Tetradecanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14f). The procedure described above (Δ) applied to $C_{13}H_{27}COOCH_2Cl$ (137 mg, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 6-35µm; MeOH/NEt_3/CH_2Cl_2 7.5/1/100) and subsequent acetone wash (10 mL) 234 mg (77%) of the title compound as a white solid. R_f 0.27 (CH_2Cl_2/MeOH, 90/10). m.p. 184ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_o-H); 1.20 – 1.37 (m, 20H, $C_{e\text{-}n}$-H);1.53 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.65 (m, 2H, C_d-H); 2.38 (s, 3H, $C_{4'a}$-H); 2.40 (t, $^3J_{H\text{-}H}$ = 7.5 Hz, 2H, C_c-H); 2.57 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.36 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.33 – 4.55 (m, 3H, C_2-H, C_3-H); 5.95 (AB system, $^2J_{H\text{-}H}$ = 5.7 Hz, 2H, C_a-H); 7.42 (d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.20 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 14.1 (C_o); 18.3 (C_{3a}); 22.7 (C_n); 24.6 (C_m); 29.1, 29.3, 29.3, 29.5, 29.6, 29.6, 29.7 ($C_{e\text{-}l}$); 31.9 (C_d); 34.0 (C_c); 46.4 ($C_{4'a}$); 50.5 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.4 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C_8); 108.0 (C_6); 123.0 (d, $^3J_{C\text{-}F}$ = 8.4 Hz, C_{7a}); 123.5 (d, $^4J_{C\text{-}F}$ = 1.4 Hz, C_{10b}); 131.9 (d, $^2J_{C\text{-}F}$ = 14.3 Hz, C_{10}); 139.6 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C\text{-}F}$ = 2.6 Hz, C_7); 172.8 (C_b). ^{19}F NMR (282 MHz): -121.0 (d, $^3J_{F\text{-}H}$ = 11.3 Hz). MS-ESI (positive mode, MeOH) *m/z* 602.5 $[M+H]^+$. Elemental Anal. ($C_{33}H_{48}FN_3O_6$); C, H, N: calcd, 65.87, 8.04, 6.98; found, 65.65, 7.79, 6.94.

(3S)-6-[(Hexadecanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14g). The procedure described above (Δ) applied to $C_{15}H_{31}COOCH_2Cl$ (152 mg, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 6-35µm; MeOH/NEt_3/CH_2Cl_2 7.5/1/100) and subsequent acetone wash (10 mL) 252 mg (79%) of the title compound as a white solid. R_f 0.28 (CH_2Cl_2/MeOH, 90/10). m.p. 182ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_q-H); 1.21 – 1.36 (m, 24H, $C_{e\text{-}p}$-H); 1.53 (d, $^3J_{H\text{-}H}$ = 6.9 Hz, 3H, C_{3a}-H); 1.65 (m, 2H, C_d-H); 2.38 (s, 3H, $C_{4'a}$-H); 2.40 (t, $^3J_{H\text{-}H}$ = 7.2 Hz, 2H, C_c-H); 2.56 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.36 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.33 – 4.55 (m, 3H, C_2-H, C_3-H); 5.96 (AB system, $^2J_{H\text{-}H}$ = 5.6 Hz, 2H, C_a-H); 7.42 (d, $^3J_{H\text{-}F}$ = 12.3 Hz, 1H,

C_8-H); 8.20 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 14.1 (C_q); 18.3 (C_{3a}); 22.7 (C_p); 24.6 (C_o); 29.1, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 29.7 ($C_{e\text{-}n}$); 31.9 (C_d); 34.0 (C_c); 46.4 ($C_{4'a}$); 50.5 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.4 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C_8); 108.0 (C_6); 123.0 (d, $^3J_{C\text{-}F}$ = 8.4 Hz, C_{7a}); 123.5 (d, $^4J_{C\text{-}F}$ = 1.5 Hz, C_{10b}); 131.9 (d, $^2J_{C\text{-}F}$ = 14.3 Hz, C_{10}); 139.6 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C\text{-}F}$ = 2.6 Hz, C_7); 172.8 (C_b). ^{19}F NMR (282 MHz): -120.9 (d, $^3J_{F\text{-}H}$ = 11.3 Hz). MS-ESI (positive mode, MeOH) *m/z* 630.7 $[M+H]^+$. Elemental Anal. ($C_{35}H_{52}FN_3O_6 \cdot 0.2H_2O$); C, H, N: calcd, 66.37, 8.34, 6.63; found, 66.29, 8.03, 6.60.

(3S)-6-[(Octadecanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14h). The procedure described above (Δ) applied to $C_{17}H_{35}COOCH_2Cl$ (166 mg, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 6-35µm; MeOH/NEt_3/CH_2Cl_2 5/1/100) and subsequent acetone wash (10 mL) 266 mg (81 %) of the title compound as a white solid. R_f 0.29 (CH_2Cl_2/MeOH, 90/10). m.p. 180ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_s-H); 1.20 – 1.36 (m, 28H, $C_{e\text{-}r}$-H); 1.52 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.64 (m, 2H, C_d-H); 2.39 (s, 3H, $C_{4'a}$-H); 2.40 (t, $^3J_{H\text{-}H}$ = 7.5 Hz, 2H, C_c-H); 2.58 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.37 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.33 – 4.57 (m, 3H, C_2-H, C_3-H); 5.95 (AB system, $^2J_{H\text{-}H}$ = 5.7 Hz, 2H, C_a-H); 7.39 (d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.18 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 14.1 (C_s); 18.3 (C_{3a}); 22.7 (C_r); 24.6 (C_q); 29.1, 29.3, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7 ($C_{e\text{-}p}$); 31.9 (C_d); 34.0 (C_c); 46.3 ($C_{4'a}$); 50.4 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.3 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C_8); 107.9 (C_6); 123.0 (d, $^3J_{C\text{-}F}$ = 8.4 Hz, C_{7a}); 123.5 (d, $^4J_{C\text{-}F}$ = 1.4 Hz, C_{10b}); 131.8 (d, $^2J_{C\text{-}F}$ = 14.3 Hz, C_{10}); 139.6 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C\text{-}F}$ = 245.8 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C\text{-}F}$ = 2.7 Hz, C_7); 172.8 (C_b). ^{19}F NMR (282 MHz): -121.0 (d, $^3J_{F\text{-}H}$ = 11.3 Hz). MS-ESI (positive mode, MeOH) *m/z* 658.5 $[M+H]^+$. Elemental Anal. ($C_{37}H_{56}FN_3O_6 \cdot 0.5H_2O$); C, H, N: calcd, 66.64, 8.62, 6.30; found, 66.53, 8.22, 6.22.

(3S)-6-[(Eicosanoyloxy)methyl]-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (14i). The procedure described above (Δ) applied to $C_{19}H_{39}COOCH_2Cl$ (180 mg, 0.5 mmol) gave after purification by chromatography (30g SiO_2, 6-35µm; MeOH/NEt_3/CH_2Cl_2 7.5/1/100) and subsequent acetone wash (2 mL) 269 mg (79%) of the title compound as a white solid. R_f 0.32 (CH_2Cl_2/MeOH, 90/10). m.p. 181ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_u-H); 1.19 – 1.36 (m, 32H, $C_{e\text{-}t}$-H); 1.53 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.65 (m, 2H, C_d-H); 2.38 (s, 3H, $C_{4'a}$-H); 2.40 (t, $^3J_{H\text{-}H}$ = 7.5 Hz, 2H, C_c-H); 2.57 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.36 (m, 4H, $C_{2'}$-H, $C_{6'}$-H); 4.31 – 4.56

(m, 3H, C_2-H, C_3-H); 5.96 (AB system, $^2J_{H\text{-}H}$ = 5.7 Hz, 2H, C_a-H); 7.44 (d, $^3J_{H\text{-}F}$ = 12.3 Hz, 1H, C_8-H); 8.20 (s, 1H, C_5-H). ^{13}C NMR (75 MHz): 14.1 (C_u); 18.3 (C_{3a}); 22.7 (C_t); 24.6 (C_s); 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.7, 29.7 ($C_{e\text{-}r}$); 31.9 (C_d); 34.0 (C_c); 46.4 ($C_{4'a}$); 50.5 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 79.5 (C_a); 105.4 (d, $^2J_{C\text{-}F}$ = 24.1 Hz, C_8); 108.0 (C_6); 123.0 (d, $^3J_{C\text{-}F}$ = 8.5 Hz, C_{7a}); 123.5 (d, $^4J_{C\text{-}F}$ = 1.5 Hz, C_{10b}); 131.9 (d, $^2J_{C\text{-}F}$ = 14.3 Hz, C_{10}); 139.6 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 145.6 (C_5); 155.7 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C_9); 163.4 (C_{6a}); 172.5 (d, $^4J_{C\text{-}F}$ = 2.6 Hz, C_7); 172.8 (C_b). ^{19}F NMR (282 MHz): -120.9 (d, $^3J_{F\text{-}H}$ = 12.5 Hz). MS-ESI (positive mode, MeOH) *m/z* 686.5 $[M+H]^+$. Elemental Anal. ($C_{39}H_{60}FN_3O_6 \cdot 0.4H_2O$); C, H, N: calcd, 67.58, 8.84, 6.06; found, 67.47, 8.71, 6.02.

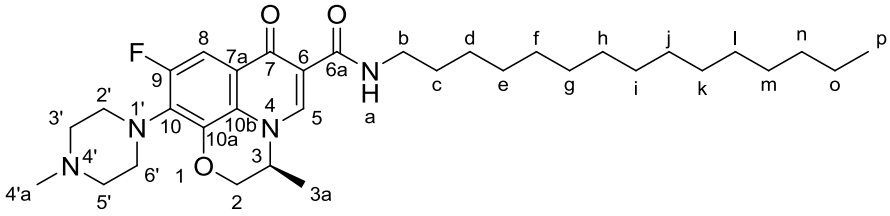


Figure 59 Atom numbering for LEV "mono-amides"

General procedure for the synthesis of (3S)-N-Alkyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamides termed LEV "mono-amides" (17a–j). To a cooled (0 ºC) and stirred mixture of LEV (361 mg, 1 mmol) and TCA (0.2 mL, 2 mmol) in 5 mL of dry CH_2Cl_2 was added dropwise, under argon, a solution of TPP (525 mg, 2 mmol) in 1 mL of dry CH_2Cl_2. The reaction mixture was stirred for 4 h at r.t. to give a yellow residue which was centrifugated, washed with CH_2Cl_2 (5 mL) and used without further purification. A suspension of this acyl chloride in 1 mL of dry CH_2Cl_2 was then treated with alkylamine (1 mmol) followed by triethylamine (303 mg, 3 mmol). The reaction mixture was allowed to react for 18 h at r.t. it was diluted to 25 mL with CH_2Cl_2 and washed with brine (3 × 50 mL). The organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure to provide after flash chromatography on silica gel (30g SiO_2, 20-45µm; MeOH/CH_2Cl_2 7.5/100) the desired compound.

(3S)-N-Butyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (17a). With $C_4H_9NH_2$ (59 mg, 1 mmol) the procedure described above provided after purification 143mg (34%) of the title compound as a yellowish solid. R_f 0.14 (CH_2Cl_2/MeOH, 90/10). m.p. 160ºC. ^{1}H NMR (300 MHz): 0.95 (t, $^3J_{H\text{-}H}$ = 7.4 Hz, 3H, C_e-H); 1.37 – 1.51 (m, 2H, C_d-H); 1.57 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.58 (m,

2H, C_c-H); 2.36 (s, 3H, $C_{4'a}$-H); 2.56 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.28 – 3.51 (m, 6H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.26 – 4.74 (m, 3H, C_2-H, C_3-H); 7.69 (d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.63 (s, 1H, C_5-H), 9.97 (t, $^3J_{H\text{-}H}$ = 5.6 Hz, 1H, N-H). ^{13}C NMR (75 MHz, $CDCl_3$): 13.8 (C_e); 18.2 (C_{3a}); 20.3 (C_d); 31.7 (C_c); 38.9 (C_b), 46.4 ($C_{4'a}$); 50.6 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.2 (C_2); 105.1 (d, $^2J_{C\text{-}F}$ = 23.9 Hz, C_8); 111.3 (C_6); 122.4 (d, $^3J_{C\text{-}F}$ = 8.7 Hz, C_{7a}); 124.3 (d, $^4J_{C\text{-}F}$ = 1.3 Hz, C_{10b}); 131.8 (d, $^2J_{C\text{-}F}$ = 14.6 Hz, C_{10}); 139.4 (d, $^3J_{C\text{-}F}$ = 6.7 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^1J_{C\text{-}F}$ = 245.8 Hz, C_9); 164.8 (C_{6a}); 175.4 (d, $^4J_{C\text{-}F}$ = 3.0 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^3J_{F\text{-}H}$ = 12.4 Hz). MS-ESI (positive mode, MeOH) *m/z* 417.3 $[M+H]^+$. Elemental Anal. ($C_{22}H_{29}FN_4O_3 \cdot 0.3H_2O$); C, H, N: calcd, 62.63, 7.07, 13.28; found, 62.59, 6.99, 13.09.

(3S)-N-Hexyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (17b). With $C_6H_{13}NH_2$ (101 mg, 1 mmol) the procedure described above provided after purification 166 mg (37%) of the title compound as a yellowish solid. R_f 0.15 (CH_2Cl_2/MeOH, 90/10). m.p. 113ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^3J_{H\text{-}H}$ = 6.9 Hz, 3H, C_g-H); 1.25 – 1.47 (m, 6H, $C_{d\text{-}f}$-H); 1.57 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.62 (m, 2H, C_c-H); 2.36 (s, 3H, $C_{4'a}$-H); 2.55 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.28 – 3.49 (m, 6H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.27 – 4.48 (m, 3H, C_2-H, C_3-H); 7.68 (d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.64 (s, 1H, C_5-H), 9.96 (t, $^3J_{H\text{-}H}$ = 5.4 Hz, 1H, N-H). ^{13}C NMR (75 MHz): 14.1 (C_g); 18.3 (C_{3a}); 22.6, 26.8, 29.6, 31.6 ($C_{c\text{-}f}$); 39.3 (C_b), 46.4 ($C_{4'a}$); 50.6 (d, $^4J_{C\text{-}F}$ = 3.8 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.2 (C_2); 105.1 (d, $^2J_{C\text{-}F}$ = 23.3 Hz, C_8); 111.3 (C_6); 122.4 (d, $^3J_{C\text{-}F}$ = 8.3 Hz, C_{7a}); 124.3 (d, $^4J_{C\text{-}F}$ = 1.5 Hz, C_{10b}); 131.8 (d, $^2J_{C\text{-}F}$ = 14.3 Hz, C_{10}); 139.4 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^1J_{C\text{-}F}$ = 246.0 Hz, C_9); 164.8 (C_{6a}); 175.4 (d, $^4J_{C\text{-}F}$ = 3.0 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^3J_{F\text{-}H}$ = 12.4 Hz). MS-ESI (positive mode, MeOH) *m/z* 445.5 $[M+H]^+$. Elemental Anal. ($C_{24}H_{33}FN_4O_3$); C, H, N: calcd, 64.84, 7.48, 12.60; found, 64.83, 7.35, 12.57.

(3S)-N-Heptyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (17c). With $C_7H_{15}NH_2$ (115 mg, 1 mmol) the procedure described above provided after purification 184 mg (36%) as yellowish solid. R_f 0.16 (CH_2Cl_2/MeOH, 90/10). m.p. 115 °C. ^{1}H NMR (300 MHz): 0.87 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_h-H); 1.20–1.45 (m, 8H, $C_{d\text{-}g}$-H); 1.57 (d, $^3J_{H\text{-}H}$ = 6.7 Hz, 3H, C_{3a}–H); 1.63 (m, 2H, C_c-H); 2.36 (s, 3H, $C_{4'a}$-H); 2.54 (m, 4H, $C_{3'}$-H and $C_{5'}$-H); 3.28–3.50 (m, 6H, C_b-H, $C_{2'}$-H and $C_{6'}$-H); 4.27–4.48 (m, 3H, C_2-H and C_3-H); 7.68 (d, $^3J_{H\text{-}F}$ = 12.5 Hz, 1H, C_8-H); 8.64 (s, 1H, C_5-H); 9.98 (t, $^3J_{H\text{-}H}$ = 5.4 Hz, 1H, N–H). ^{13}C NMR (75 MHz): 14.1 (C_h); 18.2 (C_{3a}); 22.6, 27.1, 29.0, 29.6, 31.7, 39.3 ($C_{c\text{-}g}$); 46.4 ($C_{4'a}$); 50.6 (d, $^4J_{C\text{-}F}$ = 3.8 Hz, $C_{2'}$ and $C_{6'}$); 54.8 (C_3); 55.7 ($C_{3'}$ and $C_{5'}$); 68.2 (C_2); 105.1 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C_8); 111.3 (C_6); 122.4 (d, $^3J_{C\text{-}F}$ = 9.0 Hz, C_{7a}); 124.3 (d, $^4J_{C\text{-}F}$ = 1.5 Hz,

C_{10b}); 131.8 (d, $^{2}J_{C\text{-}F}$ = 14.3 Hz, C_{10}); 139.4 (d, $^{3}J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^{1}J_{C\text{-}F}$ = 246.0 Hz, C_9); 164.8 (C_{6a}); 175.4 (d, $^{4}J_{C\text{-}F}$ = 3.0 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^{3}J_{H\text{-}F}$ = 12.5 Hz). MS (ESI, positive mode): *m/z* 459.1 [M+H]+. Elemental Anal. ($C_{25}H_{35}FN_4O_3$); C, H, N: 65.48, 7.69, 12.22; found, 65.41, 7.47, 12.22.

(3S)-N-Octyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (17d). With $C_8H_{17}NH_2$ (129 mg, 1 mmol) the procedure described above provided after purification 168 mg (36%) of the title compound as a yellowish solid. R_f 0.17 (CH_2Cl_2/MeOH, 90/10). m.p. 122ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^{3}J_{H\text{-}H}$ = 6.8 Hz, 3H, C_i-H); 1.21 – 1.47 (m, 10H, $C_{d\text{-}h}$-H); 1.58 (d, $^{3}J_{H\text{-}H}$ = 6.9 Hz, 3H, C_{3a}-H); 1.63 (m, 2H, C_c-H); 2.37 (s, 3H, $C_{4'a}$-H); 2.56 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.29 – 3.51 (m, 6H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.26 – 4.47 (m, 3H, C_2-H, C_3-H); 7.71 (d, $^{3}J_{H\text{-}F}$ = 12.3 Hz, 1H, C_8-H); 8.64 (s, 1H, C_5-H), 9.98 (t, $^{3}J_{H\text{-}H}$ = 5.6 Hz, 1H, N-H). ^{13}C NMR (75 MHz): 14.1 (C_i); 18.2 (C_{3a}); 22.7, 27.2, 29.2, 29.3, 29.7, 31.8 ($C_{c\text{-}h}$); 39.3 (C_b), 46.4 ($C_{4'a}$); 50.6 (d, $^{4}J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.2 (C_2); 105.1 (d, $^{2}J_{C\text{-}F}$ = 24.0 Hz, C_8); 111.3 (C_6); 122.5 (d, $^{3}J_{C\text{-}F}$ = 8.7 Hz, C_{7a}); 124.3 (d, $^{4}J_{C\text{-}F}$ = 1.3 Hz, C_{10b}); 131.8 (d, $^{2}J_{C\text{-}F}$ = 14.6 Hz, C_{10}); 139.4 (d, $^{3}J_{C\text{-}F}$ = 6.7 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^{1}J_{C\text{-}F}$ = 245.9 Hz, C_9); 164.8 (C_{6a}); 175.4 (d, $^{4}J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^{3}J_{F\text{-}H}$ = 11.3 Hz). MS-ESI (positive mode, MeOH) *m/z* 473.2 $[M+H]^+$. Elemental Anal. ($C_{26}H_{37}FN_4O_3 \cdot 0.5H_2O$); C, H, N: calcd, 64.84, 7.95, 11.63; found, 64.87, 7.72, 11.67.

(3S)-N-Decyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (17e). With $C_{10}H_{21}NH_2$ (157 mg, 1 mmol) the procedure described above provided after purification 176 mg (35%) of the title compound as a yellowish solid. R_f 0.17 (CH_2Cl_2/MeOH, 90/10). m.p. 133ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^{3}J_{H\text{-}H}$ = 6.6 Hz, 3H, C_k-H); 1.19 – 1.47 (m, 14H, $C_{d\text{-}j}$-H); 1.58 (d, $^{3}J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.64 (m, 2H, C_c-H); 2.37 (s, 3H, $C_{4'a}$-H); 2.56 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.29 – 3.50 (m, 6H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.27 – 4.46 (m, 3H, C_2-H, C_3-H); 7.71 (d, $^{3}J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.64 (s, 1H, C_5-H), 9.98 (t, $^{3}J_{H\text{-}H}$ = 5.6 Hz, 1H, N-H). ^{13}C NMR (75 MHz): 14.1 (C_k); 18.2 (C_{3a}); 22.7, 27.2, 29.3, 29.4, 29.5, 29.6, 29.6, 31.9 ($C_{c\text{-}j}$); 39.3 (C_b), 46.4 ($C_{4'a}$); 50.6 (d, $^{4}J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.2 (C_2); 105.1 (d, $^{2}J_{C\text{-}F}$ = 24.0 Hz, C_8); 111.3 (C_6); 122.5 (d, $^{3}J_{C\text{-}F}$ = 8.7 Hz, C_{7a}); 124.3 (d, $^{4}J_{C\text{-}F}$ = 1.3 Hz, C_{10b}); 131.8 (d, $^{2}J_{C\text{-}F}$ = 14.5 Hz, C_{10}); 139.4 (d, $^{3}J_{C\text{-}F}$ = 6.7 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^{1}J_{C\text{-}F}$ = 245.9 Hz, C_9); 164.8 (C_{6a}); 175.4 (d, $^{4}J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^{3}J_{F\text{-}H}$ = 11.3 Hz). MS-ESI (positive mode, MeOH) *m/z* 501.5

$[M+H]^+$. Elemental Anal. ($C_{28}H_{41}FN_4O_3 \cdot 0.3H_2O$); C, H, N: calcd, 66.46, 8.29, 11.07; found, 66.35, 7.97, 11.07.

(3S)-N-Dodecyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (17f). With $C_{12}H_{25}NH_2$ (185 mg, 1 mmol) the procedure described above provided after purification 197 mg (37%) of the title compound as a yellowish solid. R_f 0.19 (CH_2Cl_2/MeOH, 90/10). m.p. 101ºC. ^{1}H NMR (300 MHz): 0.88 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_m-H); 1.19 – 1.47 (m, 18H, $C_{d\text{-}l}$-H); 1.58 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.63 (m, 2H, C_c-H); 2.37 (s, 3H, $C_{4'a}$-H); 2.55 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.28 – 3.49 (m, 6H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.26 – 4.46 (m, 3H, C_2-H, C_3-H); 7.71 (d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.64 (s, 1H, C_5-H), 9.98 (t, $^3J_{H\text{-}H}$ = 5.6 Hz, 1H, N-H). ^{13}C NMR (75 MHz): 14.1 (C_m); 18.2 (C_{3a}); 22.7, 27.2, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9 ($C_{c\text{-}l}$); 39.3 (C_b), 46.4 ($C_{4'a}$); 50.6 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.2 (C_2); 105.1 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C_8); 111.3 (C_6); 122.5 (d, $^3J_{C\text{-}F}$ = 8.8 Hz, C_{7a}); 124.3 (d, $^4J_{C\text{-}F}$ = 1.2 Hz, C_{10b}); 131.8 (d, $^2J_{C\text{-}F}$ = 14.6 Hz, C_{10}); 139.4 (d, $^3J_{C\text{-}F}$ = 6.7 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C_9); 164.8 (C_{6a}); 175.4 (d, $^4J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^3J_{F\text{-}H}$ = 12.4 Hz). MS-ESI (positive mode, MeOH) *m/z* 529.6 $[M+H]^+$. Elemental Anal. ($C_{30}H_{45}FN_4O_3$); C, H, N: calcd, 68.15, 8.58, 10.60; found, 68.79, 8.06, 10.70.

(3S)-N-Tetradecyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (17h). With $C_{14}H_{29}NH_2$ (213 mg, 1 mmol) the procedure described above provided after purification 216 mg (39%) of the title compound as a yellowish solid. R_f 0.20 (CH_2Cl_2/MeOH, 90/10). m.p. 90ºC. ^{1}H NMR (300 MHz): 0.89 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C_o-H); 1.20 – 1.47 (m, 22H, $C_{d\text{-}n}$-H); 1.58 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.64 (m, 2H, C_c-H); 2.38 (s, 3H, $C_{4'a}$-H); 2.57 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.30 – 3.50 (m, 6H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.27 – 4.47 (m, 3H, C_2-H, C_3-H); 7.72 (d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.64 (s, 1H, C_5-H), 9.98 (t, $^3J_{H\text{-}H}$ = 5.6 Hz, 1H, N-H). ^{13}C NMR (75 MHz): 14.1 (C_o); 18.2 (C_{3a}); 22.7, 27.2, 29.4, 29.4, 29.5, 29.6, 29.7, 29.7, 29.7, 29.7, 31.9 ($C_{c\text{-}n}$); 39.3 (C_b), 46.4 ($C_{4'a}$); 50.6 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.2 (C_2); 105.1 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C_8); 111.3 (C_6); 122.5 (d, $^3J_{C\text{-}F}$ = 8.8 Hz, C_{7a}); 124.3 (d, $^4J_{C\text{-}F}$ = 1.3 Hz, C_{10b}); 131.8 (d, $^2J_{C\text{-}F}$ = 14.6 Hz, C_{10}); 139.4 (d, $^3J_{C\text{-}F}$ = 6.6 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C_9); 164.8 (C_{6a}); 175.4 (d, $^4J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^3J_{F\text{-}H}$ = 14.1 Hz). MS-ESI (positive mode, MeOH) *m/z* 557.6 $[M+H]^+$. Elemental Anal. ($C_{32}H_{49}FN_4O_3$); C, H, N: calcd, 69.03, 8.87, 10.06; found, 68.78, 8.67, 9.93.

(3S)-N-Pentadecyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (17i). With $C_{15}H_{31}NH_2$ (227 mg, 1 mmol) the procedure described above provided after purification 231 mg (40%) of the title compound as a yellowish solid. R_f 0.20 (CH_2Cl_2/MeOH, 90/10). m.p. 87ºC. ^{1}H NMR (300 MHz): 0. 90 (t, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_p-H); 1.21 – 1.49 (m, 24H, $C_{d\text{-}o}$-H); 1.59 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.65 (m, 2H, C_c-H); 2.38 (s, 3H, $C_{4'a}$-H); 2.57 (m, 4H, $C_{3'}$-H, $C_{5'}$-H); 3.29 – 3.52 (m, 6H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.28 – 4.49 (m, 3H, C_2-H, C_3-H); 7.72 (d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, C_8-H); 8.65 (s, 1H, C_5-H), 10.00 (t, $^3J_{H\text{-}H}$ = 5.7 Hz, 1H, N-H). ^{13}C NMR (75 MHz): 14.1 (C_p); 18.3 (C_{3a}); 22.7, 27.2, 29.4, 29.4, 29.6, 29.7, 29.7, 29.7, 29.7, 29.7, 31.9 ($C_{c\text{-}o}$); 39.3 (C_b), 46.4 ($C_{4'a}$); 50.6 (d, $^4J_{C\text{-}F}$ = 4.1 Hz, $C_{2'}$, $C_{6'}$); 54.9 (C_3); 55.7 ($C_{3'}$,$C_{5'}$); 68.2 (C_2); 105.1 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C_8); 111.3 (C_6); 122.5 (d, $^3J_{C\text{-}F}$ = 8.7 Hz, C_{7a}); 124.3 (d, $^4J_{C\text{-}F}$ = 1.3 Hz, C_{10b}); 131.9 (d, $^2J_{C\text{-}F}$ = 14.6 Hz, C_{10}); 139.4 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^1J_{C\text{-}F}$ = 245.8 Hz, C_9); 164.8 (C_{6a}); 175.4 (d, $^4J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^3J_{F\text{-}H}$ = 12.4 Hz). MS-ESI (positive mode, MeOH) *m/z* 571.6 $[M+H]^+$. Elemental Anal. ($C_{33}H_{51}FN_4O_3 \cdot 0.6H_2O$);); C, H, N: calcd, 69.44, 9.01, 9.82; found, 69.10, 8.93, 9.75.

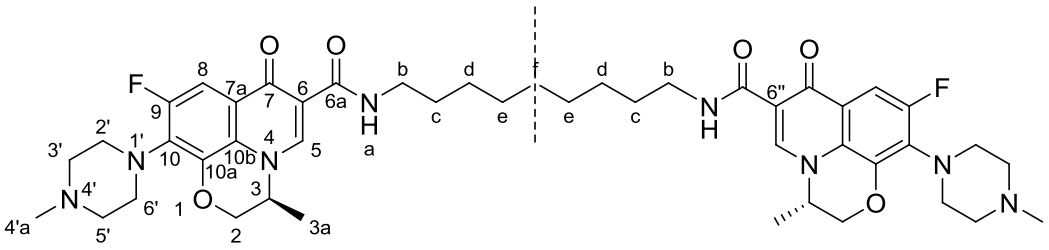


Figure 60 Atom numbering for LEV "di-amides"

General procedure for the preparation of bis((3S)-N-alkyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide termed LEV "di-amides" (18a-h). TCA (0.2 mL, 2 mmol) was added dropwise, to a solution of LEV (295 mg, 0.83 mmol) in dry CH_2Cl_2 (5 mL). Then, a solution of triphenylphosphine (511 mg, 2 mmol) in CH_2Cl_2 (1 mL) was added dropwise, and the reaction mixture was left stirring from 0°C to r.t. for 4 hours. After filtration, the yellow residue (0.8 mmol of acyl chloride) in 4 mL of dry CH_2Cl_2 was treated with diamine (0.4 mmol) followed by triethylamine (0.33 mL, 2.4 mmol). The reaction mixture was allowed to react for 18 h at r.t. The mixture was concentrated in vacuo, and the residue was washed with water. After filtration, the obtained residue was diluted with CH_2Cl_2, then diethyl ether was added to precipitate the desired compound which, after filtration, was purified by silica gel chromatography (CH_2Cl_2/MeOH 10/0.3).

bis((3S)-N-butyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide) (18a). The purification provided the desired compound (121 mg, 39%) as a pale yellow solid. ^{1}H NMR (300 MHz): 1.56 (d, $^{3}J_{H\text{-}H}$ = 6.6 Hz, 3H, C_{3a}-H); 1.74 (m, 4H, C_c-H); 2.36 (s, 6H, $C_{4'a}$-H); 2.55 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.33 – 3.51 (m, 12H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.26 – 4.31 (m, 2H, C_3-H); 4.37 – 4.41 (m, 4H, C_2-H); 7.67 (d, $^{3}J_{H\text{-}F}$ = 12.6 Hz, 2H, C_8-H); 8.61 (s, 2H, C_5-H), 9.99 (t, $^{3}J_{H\text{-}H}$ = 5.7 Hz, 2H, N-H). ^{13}C NMR (75 MHz): 18.2 (C_{3a}); 27.2 (C_c); 38.8 (C_b), 46.3 ($C_{4'a}$); 50.4 (d, $^{4}J_{C\text{-}F}$ = 4.0 Hz, $C_{2'}$, $C_{6'}$); 54.7 (C_3); 55.6 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 104.9 (d, $^{2}J_{C\text{-}F}$ = 23.9 Hz, C_8); 111.1 (C_6); 122.3 (d, $^{3}J_{C\text{-}F}$ = 8.8 Hz, C_{7a}); 124.2 (d, $^{4}J_{C\text{-}F}$ = 0.9 Hz, C_{10b}); 131.6 (d, $^{2}J_{C\text{-}F}$ = 14.5 Hz, C_{10}); 139.3 (d, $^{3}J_{C\text{-}F}$ = 6.7 Hz, C_{10a}); 143.7 (C_5); 155.6 (d, $^{1}J_{C\text{-}F}$ = 245.7 Hz, C_9); 164.8 (C_{6a}); 175.2 (d, $^{4}J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^{3}J_{F\text{-}H}$ = 12.4 Hz). MS CI (DCI/NH_3): *m/z* 775.4 $[M+H]^+$. Elemental Anal. ($C_{40}H_{48}F_2N_8O_6 \cdot 1.5\ H_2O$); C, H, N: calcd, 59.91, 6.41, 13.97; found, 60.05, 6.16, 13.82.

bis((3S)-N-pentyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide) (18b). The purification provided the desired compound (91 mg, 29%) as a pale yellow solid. ^{1}H NMR (300 MHz): 1.24 (m, 2H, C_d-H); 1.56 (d, ^{3}J = 6.6 Hz, 6H, C_{3a}-H); 1.68 (tt, ^{3}J = 7.2 Hz, 4H, C_c-H); 2.38 (s, 6H, $C_{4'a}$-H); 2.58 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.33 – 3.47 (m, 12H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.26 – 4.31 (m, 2H, C_3); 4.36 – 4.42 (m, 4H, C_2); 7.68 (d, $^{3}J_{H\text{-}F}$ = 12.6 Hz, 2H, C_8); 8.62 (s, 2H, C_5); 9.97 (t, ^{3}J = 5.7 Hz, 2H, NH). ^{13}C NMR (75 MHz): 18.2 (C_{3a}); 24.6, 29.3, 39.1 ($C_{b\text{-}d}$); 46.3 ($C_{4'a}$); 50.5 (d, $^{4}J_{C\text{-}F}$ = 3.9 Hz, $C_{2'}$,$C_{6'}$); 54.7 (C_3); 55.6 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 105.0 (d, $^{2}J_{C\text{-}F}$ = 24 Hz, C_8); 111.1 (C_6); 122.3 (d, $^{3}J_{C\text{-}F}$ = 8.8 Hz, C_{7a}); 124.2 (d, $^{4}J_{C\text{-}F}$ = 0.9 Hz, C_{10b}); 131.6 (d, $^{2}J_{C\text{-}F}$ = 14.6 Hz, C_{10}); 139.3 (d, $^{3}J_{C\text{-}F}$ = 6.7 Hz, C_{10a}); 143.7 (C_5); 155.7 (d, $^{1}J_{C\text{-}F}$ = 245.7 Hz, C_9); 164.8 (C_{6a}); 175.2 (d, $^{4}J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^{3}J_{H\text{-}F}$ = 12.4 Hz). MS CI (DCI/NH_3): *m/z* 789.3 $[M+H]^+$. Elemental Anal. ($C_{41}H_{50}F_2N_8O_6 \cdot 2\ H_2O$); C, H, N: calcd, 59.70, 6.60, 13.58; found, 59.67, 6.53, 13.40.

bis((3S)-N-hexyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide) (18c). The purification provided the desired compound (132 mg, 41%) as a pale yellow solid. ^{1}H NMR (300 MHz): 1.43 (m, 4H, C_d-H); 1.53 (d, $^{3}J_{H\text{-}H}$ = 6.9 Hz, 6H, C_{3a}-H); 1.63 (tt, ^{3}J = 6.6 Hz, 4H, C_c-H); 2.33 (s, 6H, $C_{4'a}$-H); 2.52 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.30 – 3.44 (m, 12H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.25 – 4.29 (m, 2H, C_3-H); 4.36 – 4.42 (m, 4H, C_2-H); 7.65 (d, $^{3}J_{H\text{-}F}$ = 12.6 Hz, 2H, C_8-H); 8.60 (s, 2H, C_5-H), 9.94 (t, $^{3}J_{H\text{-}H}$ = 5.7 Hz, 2H, N-H). ^{13}C NMR (75 MHz): 18.2 (C_{3a}); 26.8 (C_d); 29.5 (C_c); 39.1 (C_b); 46.3 ($C_{4'a}$);

50.5 (d, $^{4}J_{C\text{-}F}$ = 4.0 Hz, $C_{2'}$, $C_{6'}$); 54.7 (C_3); 55.6 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 105.0 (d, $^{2}J_{C\text{-}F}$ = 24.0 Hz, C_8); 111.2 (C_6); 122.4 (d, $^{3}J_{C\text{-}F}$ = 8.7 Hz, C_{7a}); 124.2 (d, $^{4}J_{C\text{-}F}$ = 1.0 Hz, C_{10b}); 131.6 (d, $^{2}J_{C\text{-}F}$ = 14.6 Hz, C_{10}); 139.3 (d, $^{3}J_{C\text{-}F}$ = 6.6 Hz, C_{10a}); 143.7 (C_5); 155.7 (d, $^{1}J_{C\text{-}F}$ = 245.7 Hz, C_9); 164.7 (C_{6a}); 175.2 (d, $^{4}J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.3 (d, $^{3}J_{F\text{-}H}$ = 12.4 Hz). MS CI (DCI/NH_3): *m/z* 803.3 $[M+H]^+$. Elemental Anal ($C_{42}H_{52}F_2N_8O_6 \cdot 1.5\ H_2O$); C, H, N: calcd, 60.78, 6.68, 13.50; found, 60.43, 6.29, 13.51.

bis((3S)-N-heptyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide) (18d). The purification provided the desired compound (120 mg, 37%) as a pale yellow solid. ^{1}H NMR (300 MHz): 1.40 (m, 6H, $C_{d,e}$-H); 1.56 (d, ^{3}J = 6.9 Hz, 6H, C_{3a}); 1.63 (tt, ^{3}J = 6.6 Hz, 4H, C_c-H); 2.37 (s, 6H, $C_{4'a}$); 2.56 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.31 – 3.46 (m, 12H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.26 – 4.31 (m, 2H, C_3-H); 4.37 – 4.41 (m, 4H, C_2-H); 7.69 (d, $^{3}J_{H\text{-}F}$ = 12.6 Hz, 2H, C_8-H); 8.62 (s, 2H, C_5-H); 9.94 (t, ^{3}J = 5.7 Hz, 2H, NH). ^{13}C NMR (75 MHz): 18.2 (C_{3a}); 26.9, 29, 29.5, 39.2 ($C_{b\text{-}e}$); 46.3 ($C_{4'a}$); 50.5 (d, $^{4}J_{C\text{-}F}$ = 4 Hz, $C_{2'}$, $C_{6'}$); 54.7 (C_3); 55.6 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 105.0 (d, $^{2}J_{C\text{-}F}$ = 23.9 Hz, C_8); 111.2 (C_6); 122.4 (d, $^{3}J_{C\text{-}F}$ = 8.8 Hz, C_{7a}); 124.2 (d, $^{4}J_{C\text{-}F}$ = 1.0 Hz, C_{10b}); 131.7 (d, $^{2}J_{C\text{-}F}$ = 14.6 Hz, C_{10}); 139.3 (d, $^{3}J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 143.7 (C_5); 155.7 (d, $^{1}J_{C\text{-}F}$ = 245.7 Hz, C_9); 164.7 (C_{6a});175.2 (d, $^{4}J_{C\text{-}F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.3 (d, $^{3}J_{H\text{-}F}$ = 12.4 Hz). MS CI (DCI/NH_3): m/z 817.4 $[M+H]^+$. Elemental Anal. ($C_{43}H_{54}F_2N_8O_6 \cdot 1\ H_2O$); C, H, N: calcd, 61.86, 6.76, 13.42; found, 61.52, 6.69, 13.21.

bis((3S)-N-octyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide) (18e). The purification provided the desired compound (67 mg, 23%) as a pale yellow solid. ^{1}H NMR (300 MHz): 1.28 (m, 8H, C_d-H, C_e-H); 1.56 (d, $^{3}J_{H\text{-}H}$ = 6.6 Hz, 6H, C_{3a}-H); 1.61 (tt, ^{3}J = 7.2 Hz, 4H, C_c-H); 2.39 (s, 6H, $C_{4'a}$-H); 2.59 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.33 – 3.45 (m, 12H, $C_{2'}$-H, $C_{6'}$-H, C_b-H); 4.26 – 4.30 (m, 2H, C_3-H); 4.37 – 4.41 (m, 4H, C_2-H); 7.69 (d, $^{3}J_{H\text{-}F}$ = 12.3 Hz, 2H, C_8-H); 8.62 (s, 2H, C_5-H), 9.94 (t, $^{3}J_{H\text{-}H}$ = 5.7 Hz, 2H, N-H). ^{13}C NMR (75 MHz): 18.2 (C_{3a}); 27.1, 29.3 (C_d, C_e); 29.6 (C_c); 39.3 (C_b), 46.2 ($C_{4'a}$); 50.4 (d, $^{4}J_{C\text{-}F}$ = 3.8 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_3); 55.6 ($C_{3'}$,$C_{5'}$); 68.2 (C_2); 105.1 (d, $^{2}J_{C\text{-}F}$ = 24.1 Hz, C_8); 111.4 (C_6); 122.6 (d, $^{3}J_{C\text{-}F}$ = 9.0 Hz, C_{7a}); 124.3 (d, $^{4}J_{C\text{-}F}$ = 1.0 Hz, C_{10b}); 131.6 (d, $^{2}J_{C\text{-}F}$ = 14.7 Hz, C_{10}); 139.4 (d, $^{3}J_{C\text{-}F}$ = 6.0 Hz, C_{10a}); 143.8 (C_5); 155.8 (d, $^{1}J_{C\text{-}F}$ = 246.7 Hz, C_9); 164.8 (C_{6a}); 175.3 (d, $^{4}J_{C\text{-}F}$ = 3.0 Hz, C_7). ^{19}F NMR (282 MHz): -121.3 (d, $^{3}J_{F\text{-}H}$ = 12.7 Hz). MS CI (DCI/NH_3): *m/z* 831.4 $[M+H]^+$. Elemental Anal. ($C_{44}H_{56}F_2N_8O_6 \cdot 0.5\ H_2O$); C, H, N: calcd, 62.92, 6.84, 13.84; found, 62.74, 6.79, 13.51.

bis((3S)-N-nonyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide) (18f). The purification provided the desired compound (63 mg, 20%) as a pale yellow solid. ^{1}H NMR (300 MHz): 1.28 (m, 10H, $C_{d\text{-}f}$-H); 1.56 (d, $^{3}J_{H\text{-}H}$ = 6.6 Hz, 6H, C_{3a}-H); 1.61 (tt, ^{3}J = 6.9 Hz, 4H, C_{c}-H); 2.38 (s, 6H, $C_{4'a}$-H); 2.58 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.33 – 3.45 (m, 12H, $C_{2'}$-H, $C_{6'}$-H, C_{b}-H); 4.25 – 4.30 (m, 2H, C_{3}-H); 4.35 –4.43 (m, 4H, C_{2}-H); 7.69 (d, $^{3}J_{H\text{-}F}$ = 12.6 Hz, 2H, C_{8}-H); 8.62 (s, 2H, C_{5}-H), 9.94 (t, $^{3}J_{H\text{-}H}$ = 5.7 Hz, 2H, N-H). ^{13}C NMR (75 MHz): 18.2 (C_{3a}); 27.1, 29.4, 29.5, 29.6 ($C_{c\text{-}f}$); 39.3 (C_{b}); 46.2 ($C_{4'a}$); 50.4 (d, $^{4}J_{C\text{-}F}$ = 3.8 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_{3}); 55.6 ($C_{3'}$,$C_{5'}$); 68.2 (C_{2}); 105.1 (d, $^{2}J_{C\text{-}F}$ = 24.1 Hz, C_{8}); 111.4 (C_{6}); 122.6 (d, $^{3}J_{C\text{-}F}$ = 9.0 Hz, C_{7a}); 124.3 (d, $^{4}J_{C\text{-}F}$ = 1.0 Hz, C_{10b}); 131.6 (d, $^{2}J_{C\text{-}F}$ = 15.1 Hz, C_{10}); 139.5 (d, $^{3}J_{C\text{-}F}$ = 6.1 Hz, C_{10a}); 143.8 (C_{5}); 155.8 (d, $^{1}J_{C\text{-}F}$ = 246.7 Hz, C_{9}); 164.8 (C_{6a}); 175.3 (d, $^{4}J_{C\text{-}F}$ = 3.0 Hz, C_{7}). ^{19}F NMR (282 MHz): -121.3 (d, $^{3}J_{F\text{-}H}$ = 12.1 Hz). MS CI (DCI/NH_3): *m/z* 845.4 $[M+H]^+$. Elemental Anal. ($C_{45}H_{58}F_2N_8O_6 \cdot 1.9\ H_2O$); C, H, N: calcd, 61.47, 7.08, 12.74; found, 61.34, 6.92, 12.79.

bis((3S)-N-decyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide) (18g). The purification provided the desired compound (94 mg, 20%) as a pale yellow solid. ^{1}H NMR (300 MHz): 1.28 (m, 12H, $C_{d\text{-}f}$-H); 1.56 (d, ^{3}J = 6.6 Hz, 6H, C_{3a}-H) ; 1.62 (tt, ^{3}J = 7.1 Hz, 4H, C_{c}-H); 2.44 (s, 6H, $C_{4'a}$-H); 2.67 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.33 – 3.50 (m, 12H, $C_{2'}$-H, $C_{6'}$-H, C_{b}-H); 4.27 – 4.30 (m, 2H, C_{3}-H); 4.38 – 4.42 (m, 4H, C_{2}-H); 7.70 (d, $^{3}J_{H\text{-}F}$ = 12.3 Hz, 2H, C_{8}-H); 8.63 (s, 2H, C_{5}-H); 9.93 (t, ^{3}J = 5.7 Hz, 2H, N-H). ^{13}C NMR (75 MHz): 18.2 (C_{3a}); 27.1, 29.3, 29.5, 29.6, 39.3 ($C_{b\text{-}f}$); 46.1 ($C_{4'a}$); 50.2 (d, $^{4}J_{C\text{-}F}$ = 3.8 Hz, $C_{2'}$, $C_{6'}$); 54.8 (C_{3}); 55.6 ($C_{3'}$,$C_{5'}$); 68.2(C_{2}); 105.1 (d, $^{2}J_{C\text{-}F}$ = 24.1 Hz, C_{8}); 111.4 (C_{6}); 122.7 (d, $^{3}J_{C\text{-}F}$ = 9 Hz, C_{7a}); 124.3 (d, $^{4}J_{C\text{-}F}$ = 1.0 Hz, C_{10b}); 131.5 (d, $^{2}J_{C\text{-}F}$ = 14.3 Hz, C_{10}); 139.5 (d, $^{3}J_{C\text{-}F}$ = 6.8 Hz, C_{10a}); 143.8 (C_{5}); 155.7 (d, $^{1}J_{C\text{-}F}$ = 245.7 Hz, C_{9}); 164.8 (C_{6a});175.3 (d, $^{4}J_{C\text{-}F}$ = 3 Hz, C_{7}). ^{19}F NMR (282 MHz): -121.4 (d, $^{3}J_{H\text{-}F}$ = 11.8 Hz). MS (DCI/NH_3): *m/z* 859.4 $[M+H]^+$. Elemental Anal. ($C_{46}H_{60}F_2N_8O_6 \cdot 1.5\ H_2O$); C, H, N: calcd, 62.36, 7.17, 12.65; found, 62.31, 7.02, 12.52.

bis((3S)-N-dodecyl-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide) (18h). The purification provided the desired compound (75 mg, 27%) as a pale yellow solid. ^{1}H NMR (300 MHz): 1.25 (m, 16H, $C_{d\text{-}g}$-H); 1.56 (d, ^{3}J = 6.6 Hz, 6H, C_{3a}-H); 1.62 (tt, ^{3}J = 6.9 Hz, 4H, C_{c}-H); 2.44 (s, 6H, $C_{4'a}$-H); 2.66 (m, 8H, $C_{3'}$-H, $C_{5'}$-H); 3.39 – 3.46 (m, 12H, $C_{2'}$-H, $C_{6'}$-H, C_{b}-H), 4.26 – 4.31 (m, 2H, C_{3}-H); 4.38 – 4.42 (m, 4H, C_{2}-H); 7.70 (d, $^{3}J_{H\text{-}F}$ = 12.3 Hz, 2H, C_{8}-H); 8.63 (s, 2H, C_{5}-H); 9.93 (t, ^{3}J = 6.0 Hz, 2H, N-H). ^{13}C NMR (75 MHz): 18.2 (C_{3a}); 27.1, 29.3, 29.4, 29.6, 29.6, 39.2 ($C_{b\text{-}g}$); 46.3

($C_{4'a}$); 50.5 (d, $^{4}J_{C-F}$ = 4 Hz, $C_{2'}$, $C_{6'}$); 54.7 (C_3); 55.6 ($C_{3'}$,$C_{5'}$); 68.1 (C_2); 105 (d, $^{2}J_{C-F}$ = 23.9 Hz, C_8); 111.2 (C_6); 122.4 (d, $^{3}J_{C-F}$ = 8.7 Hz, C_{7a}); 124.2 (d, $^{4}J_{C-F}$ = 1.0 Hz, C_{10b}); 131.7 (d, $^{2}J_{C-F}$ = 14.6 Hz, C_{10}); 139.3 (d, $^{3}J_{C-F}$ = 6.7 Hz, C_{10a}); 143.7 (C_5); 155.7 (d, $^{1}J_{C-F}$ = 245.7 Hz, C_9); 164.7 (C_{6a}); 175.2 (d, $^{4}J_{C-F}$ = 2.9 Hz, C_7). ^{19}F NMR (282 MHz): -121.2 (d, $^{3}J_{H-F}$ = 12.4 Hz). MS (DCI/NH_3): *m/z* 887.4 $[M+H]^+$. Elemental Anal. ($C_{48}H_{64}F_2N_8O_6 \cdot 1.5\ H_2O$); C, H, N: calcd, 63.07, 7.39, 12.26; found, 63.05, 7.30, 12.07.

2. Synthesis of octadecanoyloxymethyl ester of LEV 14h

*2.1 Reaction monitoring during the synthesis of octadecanoyloxymethyl ester of LEV **14h***

HPLC analysis was performed on a Waters system (Waters Associates Inc., Milford, MA, USA) consisting of a 600 controller pump, a PDA996 diode array detector, a Waters 2420 Evaporative Light Scattering (ELS) detector, a 717 Plus autosampler and a Lisa 30 Ecrosas (ICS) oven at 30 ºC. The instrument was controlled by the Empower software. The experiments were carried out on a C18 Luna (Phenomenex®) reverse-phase column (length 100 × 3 mm i.d., 3 μm particle size) eluted with a mobile phase consisting of various linear gradients of Solvent A (MeOH + 0.1% TFA)/Solvent B (water + 0.1% TFA) at a flow rate of 0.6 mL/min. The eluting solvents were prepared daily and degassed with helium during analyses. The detection was set at 325 nm. In a typical experiment 100μl of reaction mixture was diluted with $CHCl_3$ (HPLC grade) to a final volume of 5 mL and analyzed by HPLC (5 μl injection volume).

2.1.1 HPLC Conditions

The mobile phase consisted of linear gradient presented in **Table 83**. Waters 2420 Evaporative Light Scattering (ELS) detector was used for the quantification.

Table 83 Mobile phase composition for reaction monitoring

Time, min	*A*[a], %	*B*[b], %
0	20	80
1	20	80
5	0	100
20	0	100
21	20	80
25	20	80

[a]0.1% trifluoroacetic acid in water [b]0.1% trifluoroacetic acid in methanol

2.1.2 Calibration

Compound **14h** and LEV were dissolved in the appropriate volume $CHCl_3$ according to **Table 84** to produce the final solutions.

Table 84 Calibration solution preparation for reaction monitoring

Ref	M_w	*mass, mg*	$CHCl_3$ *volume, mL*	*Final concentration, mg/mL*
LEV	361.4	19.30	50	0.3860
14h	657.9	40.11	50	0.8022

Each compound in $CHCl_3$ solution (final solution from **Table 84**) was mixed with appropriate volume of $CHCl_3$, according to **Table 85**, to yield 4 separate standard solutions (four different concentrations per compound) which were further used to build the calibration curves. Two injections were made for each concentration.

Table 85 Standard solutions for reaction monitoring

№	*Compound solution volume*	$CHCl_3$ *volume*	*Degree of dilution*
1	1 mL	–	1.000
2	2 mL	1 mL	0.6666
3	1 mL	2 mL	0.3333
4	1 mL	9 mL	0.1000

Separate standard solutions were injected using appropriate gradient (**Table 83**). Analytical data were processed by the Empower® software, leading to the corresponding equation:

Peak area (LS Units · sec) = A + B × concentration (mg/mL) + C × concentration^2 (**Table 86**)

Table 86 Calibration parameters for reaction monitoring

Reference	*Retention time, min*	$A \cdot 10^{-3}$	$B \cdot 10^{-6}$	$C \cdot 10^{-6}$	R^2
LEV	0.971	5.91	1.39	14.0	0.9987
14h	5.816	-270	5.12	9.05	0.9997

2.1.3 Reaction monitoring procedure

47.25 mg (0.096 mmol) of cesium salt of levofloxacin and 46.46 mg (0.139 mmol) of chloromethyloctadecanoate **10h** were dissolved in 4.85 mL of dry DMF (H_2O < 6ppm) and the resulted solution was stirred at 90ºC. During the first hour four consequent probes of the reaction mixture were taken and analyzed (0 min, 20 min, 40 min and 60 min probe). Each probe consisted of 100μL aliquot which was further diluted by $CHCl_3$ to the final volume of 5 mL (analyzed and quantified by HPLC, **Table 87**) and 50μL aliquot which was further diluted by $CDCl_3$ to the final volume of 0.6 mL (analyzed by NMR). Then another two probes (50μL aliquot) were taken at 2h and 3h time points and analyzed by NMR. After 3h of stirring the solvent was removed under reduced pressure. The solid residue was dissolved in 25mL of $CHCl_3$ (Solution 1) and 1mL of it was diluted by $CHCl_3$ to the final volume of 10mL (Solution 2). Solution 2 was analyzed by HPLC in order to prove the reliability of HPLC data in the estimation of the yield. The solvent was removed from the rest 24mL of Solution 1 under reduced pressure and the solid residue was purified by column chromatography on the silica gel (20g SiO_2, 6-35μm; CH_2Cl_2/MeOH, 10/1) to yield 31 mg (m_{isol}) of octadecanoyloxymethyl ester of LEV **14h**.

Table 87 HPLC analysis of aliquots during the reaction monitoring

Probe	*Concentration, mg/mL*	
	14h	*LEV*
20 min	0.169	0.067
40 min	0.173	0.066
60 min	0.173	0.065

2.1.4 Calculation of isolated and determined by HPLC yields

The theoretical amount of octadecanoyloxymethyl ester of LEV **14h** if no aliquots were taken: $m_{theor\ 100\%}$ = 63mg.

To calculate the theoretical amount of octadecanoyloxymethyl ester of LEV **14h** which is left after all aliquots, we have to take into account the total volume of aliquots taken (100μL × 4 + 50μL × 6 = 0.7mL) and the 1mL of Solution 1 taken away for HPLC analysis: $m_{theor} = m_{theor\ 100\%} \times (4.85mL - 0.7mL)/4.85mL \times (24/25) = 51.8mg$.

So the **isolated yield** $m_{isol}/m_{theor} \times 100\%$ = **60%**.

After the HPLC analysis of Solution 2 we have obtained the following concentration of octadecanoyloxymethyl ester of LEV **14h**: 0.139 mg/mL. So the theoretical amount of octadecanoyloxymethyl ester of LEV **14h**, in the initial reaction mixture after 3h, without taking into account the aliquots taken and according to the HPLC data was the following: m_{HPLC} = 0.139 mg/mL × 250 mL × 4.85mL/(4.85mL – 0.7mL) = 40.6mg.

So the **yield determined by HPLC** $m_{HPLC} / m_{theor\ 100\%} \times 100\%$ = **64%**.

*2.2 Reaction optimization experiments for the synthesis of octadecanoyloxymethyl ester of LEV **14h***

HPLC analysis was performed on the previously mentioned Waters system, column and solvent composition. The detection was set at 325 nm for LEV derivatives. In a typical experiment the solvent was removed from reaction mixture and a solid residue was dissolved in 50 ml of $CHCl_3$ (HPLC Grade). The resulting solution was further analyzed by HPLC (5 μL injection volume).

2.2.1 HPLC Conditions

The mobile phase consisted of linear gradient presented in **Table 88**. PDA996 diode array detector was used for the quantification.

Table 88 Mobile phase composition for reaction optimization

Time, min	*A[a], %*	*B[b], %*
0	20	80
8	20	80
12	0	100
18	0	100
19	20	80
23	20	80

[a]0.1% trifluoroacetic acid in water [b]0.1% trifluoroacetic acid in methanol

2.2.2 Calibration

The standard solutions described in the previous section (**Table 84, Table 85**) were reinjected using appropriate gradient **Table 88**. Analytical data were processed by the Empower® software, leading to the corresponding equation:

Peak area (μV · sec) = A + B × concentration (μmol) (**Table 89**)

Table 89 Calibration parameters for reaction optimization

Reference	*Retention time, min*	*λ, nm*	*A · 10^{-5}*	*B · 10^{-7}*	*R^2*
LEV	0.816	325	2.413	1.555	0.9994
14h	8.206	325	-3.167	1.017	0.9993

2.2.3 Reaction optimization procedure

During the series of small-scale experiments the correspondent amount of cesium salt of LEV and chloromethyl octadecanoate **10h** were dissolved in 2ml of appropriate solvent and were stirred for the fixed number of hours at 90ºC (**Table 90**).

Table 90 Conditions for reaction optimization experiments

Entry	*Cesium salt of LEV, mg*	***10h***		*Solvent*	*Time*
		Mass, mg	*Equivalent*		
1	27.16	27.16	1.48	CH_3CN	3h
2	21.11	21.83	1.5	DMF	1h
3	24.24	16.83	1.02	DMF	1h

In each experiment, the solvent was removed under reduced pressure after the reaction and the solid residue was dissolved in 50mL of $CHCl_3$. The resulted solution was injected using appropriate gradient (**Table 88**) and quantified using previously obtained regression model. The mass of octadecanoyloxymethyl ester of LEV **14h** was calculated by the following equation: concentration obtained by HPLC × 50mL;

3. Stability tests

HPLC analysis was performed on the previously mentioned Waters system, column and solvent composition. The detection was set at 292 or 325 nm for LEV derivatives, 280 or 345 nm for CIP derivatives. All injections (10 μL) were made in duplicate. In a typical experiment, 1 mL of a 1 mM solution of tested compound in DMSO was diluted with acetonitrile/phosphate buffer (pH 7.4) (50:50) to a final volume of 10 mL. An aliquot was discarded and kept at 4 ºC (0 day probe). The remaining solution placed in a sealed tube was heated in a water bath at 37 ºC for 7 days (7 day probe).

3.1 Detailed procedure for stability investigation of CIP "di-amides"

3.1.1 HPLC Conditions

The mobile phase consisted of linear gradient presented in **Table 91**.

Table 91 Mobile phase composition for CIP "di-amides" **7**

Time, min	*Gradient for* **7** *series*	
	A[a], *%*	*B*[b], *%*
0	70	30
2	70	30
7	0	100
18	0	100
20	70	30

[a]0.1% trifluoroacetic acid in water [b]0.1% trifluoroacetic acid in methanol

3.1.2 Calibration

PBS preparation: 8.0 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 were dissolved in 800 mL of water/acetonitrile mixture (50/50). Then pH was adjusted to 7.4 by 0.1M HCl and the resulted solution was diluted by water/acetonitrile to 1 L. The use of acetonitrile was necessary to obtain homogenous solutions due to the low solubility of tested compounds in PBS.

Compounds **7** were dissolved in the appropriate volume of DMSO according to **Table 92**. Resulted DMSO solutions were diluted with water/acetonitrile phosphate-buffered saline (PBS pH = 7.4) to produce the final concentrations.

Table 92 DMSO solutions for CIP "di-amides"

Ref.	M_w	*mass, mg*	*DMSO voulme, mL*	*Conc. in DMSO, μM*	*Final conc. in buffer, μM*
7b	857.0	9.43	20.0	550[a]	55
7c	885.0	8.96	10.1	1000	100
7e	913.1	10.12	11.1	1000	100

[a]Not soluble in DMSO at 1000 μM concentration.

Each compound in DMSO solution (**Table 92**) was mixed with appropriate volume of PBS, according to **Table 93**, to yield 4 separate standard solutions (four different concentrations per compound) which were further used to build the calibration curves. Two injections were made for each concentration.

Table 93 Calibration solutions for CIP "di-amides"

№	*Compound solution volume*	*PBS volume*	*Degree of dilution*
1	1 mL	–	1.000
2	2 mL	1 mL	0.6666
3	1 mL	2 mL	0.3333
4	1 mL	9 mL	0.1000

Separate standard solutions were injected using appropriate gradient (**Table 91**). Analytical data were processed by the Empower® software, leading to the corresponding equation:

Peak area ($\mu V \cdot sec$) = A + B × concentration (μmol) (**Table 94**)

Table 94 Calibration parameters for CIP "di-amides"

Reference	*Retention time, min*	*λ, nm*	$A \cdot 10^{-4}$	$B \cdot 10^{-4}$	R^2
7b	14.50	280	-5.472	7.092	0.9989
7c	15.08	345[a]	-1.168	1.211	0.9999
7e	15.45	280	1.405	6.793	0.9980

[a]**7c** peak was slightly overlapped with impurity peak. Detection wavelength was shifted to eliminate its contribution to the peak area.

3.1.3 Stability investigation procedure

Fifteen milliliters of each compound solution (final solutions from **Table 92**) were added to 20 mL glass vials which were sealed with glass stoppers, covered with teflon film, and placed on a 37º C shaker water bath and shaken gently for 7 days. Intermediate samples were taken at 3 and 5 days. All collected samples were analyzed by HPLC and quantified using previously obtained regression models.

To evaluate the rate of degradation of the tested compounds, CIP (degradation product expected for **7** was injected. This retention time was 2.6 min. For compounds whose stability was less than 100%, the ratio [compound peak area / (compound peak + degradation product peak) area] was calculated.

*3.2 Detailed procedure for stability investigation of LEV "mono-amides" **17** and "mono-acyloxymethyl esters" **14***

3.2.1 HPLC Conditions

The mobile phase consisted of linear gradient presented in **Table 95**.

Table 95 Mobile phase composition for LEV derivatives

Time, min	*Gradient for **17** series*	
	A[a], *%*	*B*[b], *%*
0	65	35
2	65	35
7	0	100
15	0	100
17	65	35

Time, min	*Gradient for **14** series*	
	A[a], *%*	*B*[b], *%*
0	65	35
2	65	35
7	0	100
18	0	100
20	65	35

[a]0.1% trifluoroacetic acid in water [b]0.1% trifluoroacetic acid in methanol

3.2.2 Calibration

Compounds were dissolved in DMSO according to **Table 96.** Resulted solutions were diluted by water/acetonitrile phosphate buffer (pH = 7.4) to produce final concentration.

Table 96 DMSO solutions for LEV "mono-amides" ***17*** and "mono-acyloxymethyl esters" ***14***

Ref.	M_w	*mass, mg*	*V(DMSO), mL*	*Conc. in DMSO, μM*	*Final conc. in buffer, μM*
LEV	361.4	3.82	105.8	100	10
17a (n=3)	416.5	5.43	13.5	1000	100
17b (n=6)	444.6	5.26	11.8	1000	100
17d (n=8)	472.6	6.28	13.3	1000	100
17h(n=14)	556.8	5.62	10.1	1000	100
14a (n=2)	461.5	5.45	11.8	1000	100
14c (n=6)	517.6	5.76	11.1	1000	100
14e (n=10)	573.7	5.70	9.95	1000	50[a]
14g (n=14)	629.8	6.64	10,5	1000	10[a]

[a]Corresponding volume of DMSO was added to the final solution to maintain constant DMSO concentration (10% v/v)

Each DMSO solution was mixed with appropriate amount of PBS, according to **Table 97**, to yield separate standard solutions (four different concentrations per compound) which were further used to build the calibration curves. Two injections were made for each concentration.

Table 97 Calibration solutions for LEV "mono-amides" ***17*** and "mono-acyloxymethyl esters" ***14***

№	*Compound solution volume*	*PBS volume*	*Degree of dilution*
1	1 mL	–	1.000
2	2 mL	1 mL	0.6666
3	1 mL	2 mL	0.3333
4	1	9 mL	0.1000

Separate standard solutions were injected using appropriate gradient. Analytical data were processed by the Empower® software leading to the corresponding equation:

Peak area (μV · sec) = A + B × concentration (μmol) (**Table 98**)

Table 98 Calibration parameters for LEV "mono-amides" ***17*** and "mono-acyloxymethyl esters" ***14***

Reference	*Retention time, min*	*λ, nm*	$A \cdot 10^{-4}$	$B \cdot 10^{-4}$	R^2
LEV	2.35	292	-0.1716	3.125	0.9999
17a	11.73	292	-2.289	3.554	0.9998
17b	12.89	292	2.445	3.212	0.9998
17d	13.67	292	3.038	3.388	0.9999
17h	15.40	292	2.759	3.114	0.9998
14a	7.14	292	-3.128	3.298	0.9999
14c	12.73	292	-1.439	3.361	0.9998
14e	13.95	292	0.8696	3.247	0.9997
14g	14.95	325	-0.2231	1.387	0.9996

3.2.3 Stability investigation procedure

Fifteen milliliters of each compound solution (final solution from **Table 96**) were added to 20 mL glass vials which were sealed with rubber septum stoppers and placed on a 37º C shaker water bath and shaken gently for 7 days. Intermediate samples were taken at 3 and 5 days. All collected samples were analyzed by HPLC and quantified using previously obtained regression models.

To evaluate the rate of degradation of the tested compounds, LEV (degradation product expected for acyloxymethyl esters **14a–i** and amides **17a–j**) was injected. Under these conditions, all derivatives were totally separated with respective retention times at 2.4 min (LEV), 7.1–15.0 min (esters **14a,c,e,g**) and 11.7–15.4 min (amides **17a,b,d,h**). For compounds whose stability was less than 100%, the following ratio was calculated: stability(%) = [mean measured concentration (n = 2) at 7days /mean measured concentration (n = 2) at t0] x100.

4. Biological evaluation of synthesized derivatives

4.1 Microbiology

4.1.1 Evaluation of In Vitro Antimicrobial activities

MICs were determined by broth dilution micromethod. Briefly, 100 □L of Trypcase soy broth (Biomérieux, France) was placed in each well of a 96-well microtiter plate. Hundred microlitres of the solutions to be tested (prepared in 10% DMSO) was added to the first column. Then 2-fold dilutions were carried out from one column to the next up to column 10. Columns

11 and 12 were used as a sterility control (without product and without bacteria) and a growth control (without product, with inoculum) of the medium. Fresh bacterial suspensions were prepared at 10^8cells/mL using standard strains (*S. aureus* CIP 4.83, *E. hirae* CIP 5855, *P. aeruginosa* CIP 82.118 and *E.* CIP 53126) obtained from the Institut Pasteur Collection (France). The microplates were inoculated using a multipoint inoculator (Denley) to obtain a final concentration of 10^6cells/mL. Plates were then incubated at 37 °C for 18–24 h under aerobic conditions. MIC was determined as the lowest concentration with no visible growth. Assays were performed in duplicate.

4.1.2 In vitro inhibition of Mycobacterium Tuberculosis

Susceptibility of *M. tuberculosis* (strain H37Rv) to the synthetic compounds was tested by determining the MIC. A colorimetric microassay based on the reduction of MTT to formazan by metabolically active cells was used. Briefly, serial twofold dilutions of each compound were prepared in 7H9 broth (Middlebrook 7H9 broth base, Difco) using 96-well microtiter plates and 100 μL of M. tuberculosis suspension in 7H9 broth were added to each well. After 6 days incubation, MTT was added (50 μL, 1 mg/mL). After one day incubation, solubilization buffer was added to each well. The optical densities were measured at 570 nm. The MIC was determined as the lowest concentration of compound that inhibited bacterial growth (absorbance from untreated bacilli was taken as a control for growth).

4.2 Evaluation of in vitro cell proliferation by means of the MTT colorimetric assay

The assessment of cell population growth is based on the capability of living cells to reduce the yellow product MTT (Sigma, Bornem, Belgium) to a blue product, formazan, by a reduction reaction occurring in the mitochondria[190]. The five cell lines were incubated for 24 h in 96-microwell plates (at a concentration of 10^4 to 3 x 10^4 cells/mL culture medium depending on the cell type) to ensure adequate plating prior to cell growth determination. The number of living cells after 120 h of culture in the presence or absence (control) of the various drugs is directly proportional to the intensity of the blue color, measured by spectrophotometry using a 680XR microplate reader (Bio-Rad Laboratories Inc, Hercules, CA) at a wavelength of 570 nm (with a reference at 630 nm). Each experiment was carried out in hexaplicate. Nine concentrations ranging from 10^{-3} to 10^{-7} Mw (with semi-log decrease in concentration) were tested for each of the compounds under study.

4.3 In vivo testing maximum tolerated dose procedure

The acute MTD was determined following single i.p. administration of increasing drug doses to groups of three healthy B6D2F1 female mice (Charles River, Brussels, Belgium). Five dose levels (5, 10, 20, 40 and 80 mg/kg) of drug were evaluated. The MTD was defined as the dose just below the lowest dose level that killed at least one mouse in a treatment group after a maximum of 28 days.

BIBLIOGRAPHY

1. Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, R. P., 1,8-Naphthyridine Derivatives. A New Class of Chemotherapeutic Agents. *J. Med. Pharm. Chem.* **1962,** *5* (5) 1063-1065.

2. Wagman, A. S.; Wentland, M. P., Quinolone Antibacterial Agents. In *Comprehensive Medicinal Chemistry II*, John, B. T.; David, J. T., Eds. Elsevier: Oxford, **2007**, 567-596.

3. Oliphant, C. M.; Green, G. M., Quinolones: a comprehensive review. *Am. Fam. Physician* **2002,** *65* (3) 455-464.

4. Ward-Mcquaid, J. F.; Jichlinski, D.; Macis, R., Nalidixic Acid in Urinary Infections. *Br. Med. J.* **1963,** *2* (5368) 1311-4.

5. Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T., 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.

6. (a) Wise, R., Norfloxacin—a review of pharmacology and tissue penetration. *J. Antimicrob. Chemother.* **1984,** *13* (suppl B) 59-64; (b) Montay, G.; Goueffon, Y.; Roquet, F., Absorption, distribution, metabolic fate, and elimination of pefloxacin mesylate in mice, rats, dogs, monkeys, and humans. *Antimicrob. Agents Chemother.* **1984,** *25* (4) 463-472.

7. Wise, R.; Andrews, J. M.; Edwards, L. J., In vitro activity of Bay 09867, a new quinoline derivative, compared with those of other antimicrobial agents. *Antimicrob. Agents Chemother*. **1983,** *23* (4) 559-564.

8. Fass, R. J., In vitro activity of ciprofloxacin (Bay o 9867). *Antimicrob. Agents Chemother*. **1983,** *24* (4) 568-574.

9. (a) Crump, B.; Wise, R.; Dent, J., Pharmacokinetics and tissue penetration of ciprofloxacin. *Antimicrob. Agents Chemother.* **1983,** *24* (5) 784-786; (b) Lode, H.; Hoffken, G.; Olschewski, P.; Sievers, B.; Kirch, A.; Borner, K.; Koeppe, P., Pharmacokinetics of ofloxacin after parenteral and oral administration. *Antimicrob. Agents Chemother.* **1987,** *31* (9) 1338-1342.

10. Ball, P., Emergent resistance to ciprofloxacin amongst Pseudomonas aeruginosa and Staphylococcus aureus:clinical significance and therapeutic approaches. *J. Antimicrob. Chemother*. **1990,** *26* (suppl F) 165-179.

11. Mitscher, L. A.; Sharma, P. N.; Chu, D. T. W.; Shen, L. L.; Pernet, A. G., Chiral DNA gyrase inhibitors. 2. Asymmetric synthesis and biological activity of the enantiomers of 9-

fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (ofloxacin). *J. Med. Chem.* **1987,** *30* (12) 2283-2286.

12. Fu, K. P.; Lafredo, S. C.; Foleno, B.; Isaacson, D. M.; Barrett, J. F.; Tobia, A. J.; Rosenthale, M. E., In Vitro and In Vivo Antibacterial Activities of Levofloxacin (l-Ofloxacin), an Optically Active Ofloxacin. *Antimicrob. Agents Chemother.* **1992,** *36* (8) 860-866.

13. Dalhoff, A.; Schmitz, F. J., In Vitro Antibacterial Activity and Pharmacodynamics of New Quinolones. *Eur. J. Clin. Microbiol. Infect. Dis.* **2003,** *22* (4) 203-221.

14. Coyle, E. A.; Kaatz, G. W.; Rybak, M. J., Activities of Newer Fluoroquinolones against Ciprofloxacin-Resistant Streptococcus pneumoniae. *Antimicrob. Agents Chemother.* **2001,** *45* (6) 1654-1659.

15. Boswell, F. J.; Andrews, J. M.; Jevons, G.; Wise, R., Comparison of the in vitro activities of several new fluoroquinolones against respiratory pathogens and their abilities to select fluoroquinolone resistance. *J. Antimicrob. Chemother.* **2002,** *50* (4) 495-502.

16. Lubasch, A.; Keller, I.; Borner, K.; Koeppe, P.; Lode, H., Comparative Pharmacokinetics of Ciprofloxacin, Gatifloxacin, Grepafloxacin, Levofloxacin, Trovafloxacin, and Moxifloxacin after Single Oral Administration in Healthy Volunteers. *Antimicrob. Agents Chemother.* **2000,** *44* (10) 2600-2603.

17. (a) Spangler, S. K.; Jacobs, M. R.; Appelbaum, P. C., Activity of CP 99,219 compared with those of ciprofloxacin, grepafloxacin, metronidazole, cefoxitin, piperacillin, and piperacillin-tazobactam against 489 anaerobes. *Antimicrob. Agents Chemother.* **1994,** *38* (10) 2471-2476; (b) Goldstein, E. J. C.; Citron, D. M.; Warren, Y.; Tyrrell, K.; Merriam, C. V., In Vitro Activity of Gemifloxacin (SB 265805) against Anaerobes. *Antimicrob. Agents Chemother.* **1999,** *43* (9) 2231-2235.

18. Marshall, S. A.; Jones, R. N., In vitro activity of DU-6859a, a new fluorocyclopropyl quinolone. *Antimicrob. Agents Chemother.* **1993,** *37* (12) 2747-2753.

19. Ozaki, M.; Matsuda, M.; Tomii, Y.; Kimura, K.; Kazuno, K.; Kitano, M.; Kise, M.; Shibata, K.; Otsuki, M.; Nishino, T., In vitro antibacterial activity of a new quinolone, NM394. *Antimicrob. Agents Chemother.* **1991,** *35* (12) 2490-2495.

20. Ozaki, M.; Matsuda, M.; Tomii, Y.; Kimura, K.; Segawa, J.; Kitano, M.; Kise, M.; Shibata, K.; Otsuki, M.; Nishino, T., In vivo evaluation of NM441, a new thiazeto-quinoline derivative. *Antimicrob. Agents Chemother.* **1991,** *35* (12) 2496-2499.

21. (a) Sugino, A.; Peebles, C. L.; Kreuzer, K. N.; Cozzarelli, N. R., Mechanism of action of nalidixic acid: Purification of Escherichia coli nalA gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. *Proc. Natl. Acad. Sci. U. S. A.* **1977,** *74* (11) 4767-4771; (b) Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Itoh, T.; Tomizawa, J.-I., Nalidixic acid

resistance: A second genetic character involved in DNA gyrase activity. *Proc. Natl. Acad. Sci. U. S. A.* **1977,** *74* (11) 4772-4776.

22. (a) Kato, J.-i.; Nishimura, Y.; Imamura, R.; Niki, H.; Hiraga, S.; Suzuki, H., New topoisomerase essential for chromosome segregation in E. coli. *Cell* **1990,** *63* (2) 393-404; (b) Kato, J.; Suzuki, H.; Ikeda, H., Purification and characterization of DNA topoisomerase IV in Escherichia coli. *J. Biol. Chem.* **1992,** *267* (36) 25676-25684.

23. Ferrero, L.; Cameron, B.; Manse, B.; Lagneaux, D.; Crouzet, J.; Famechon, A.; Blanche, F., Cloning and primary structure of Staphylococcus aureus DNA topoisomerase IV: a primary target of fluoroquinolones. *Mol. Microbiol.* **1994,** *13* (4) 641-653.

24. Dong, K. C.; Berger, J. M., Structural basis for gate-DNA recognition and bending by type IIA topoisomerases. *Nature* **2007,** *450* (7173) 1201-1205.

25. Wentzell, L. M.; Maxwell, A., The Complex of DNA Gyrase and Quinolone Drugs on DNA Forms a Barrier to the T7 DNA Polymerase Replication Complex. *J. Mol. Biol.* **2000,** *304* (5) 779-791.

26. (a) Chen, C.-R.; Malik, M.; Snyder, M.; Drlica, K., DNA Gyrase and Topoisomerase IV on the Bacterial Chromosome: Quinolone-induced DNA Cleavage. *J. Mol. Biol.* **1996,** *258* (4) 627-637; (b) Pohlhaus, J. R.; Kreuzer, K. N., Norfloxacin-induced DNA gyrase cleavage complexes block Escherichia coli replication forks, causing double-stranded breaks in vivo. *Mol. Microbiol.* **2005,** *56* (6) 1416-1429.

27. Laponogov, I.; Sohi, M. K.; Veselkov, D. A.; Pan, X.-S.; Sawhney, R.; Thompson, A. W.; McAuley, K. E.; Fisher, L. M.; Sanderson, M. R., Structural insight into the quinolone-DNA cleavage complex of type IIA topoisomerases. *Nat. Struct. Mol. Biol.* **2009,** *16* (6) 667-669.

28. Pan, X.-S.; Fisher, L. M., Streptococcus pneumoniae DNA Gyrase and Topoisomerase IV: Overexpression, Purification, and Differential Inhibition by Fluoroquinolones. *Antimicrob. Agents Chemother.* **1999,** *43* (5) 1129-1136.

29. Ledoussal, B.; Almstead, J.-I. K.; Gray, J. L.; Hu, E. X.; Roychoudhury, S., Discovery, Structure-Activity Relationships and Unique Properties of Non- Fluorinated Quinolones (NFQs). *Curr. Med. Chem.: Anti-Infect. Agents* **2003,** *2* (1) 13-25.

30. EARSS annual report *http://www.rivm.nl/earss/result/Monitoring_reports/* [Online], **2001, 2005, 2008**.

31. Heisig, P., Genetic evidence for a role of parC mutations in development of high-level fluoroquinolone resistance in Escherichia coli. *Antimicrob. Agents Chemother.* **1996,** *40* (4) 879-885.

32. Yoshida, H.; Bogaki, M.; Nakamura, M.; Nakamura, S., Quinolone resistance-determining region in the DNA gyrase gyrA gene of Escherichia coli. *Antimicrob. Agents Chemother.* **1990,** *34* (6) 1271-1272.

33. Yoshida, H.; Bogaki, M.; Nakamura, M.; Yamanaka, L. M.; Nakamura, S., Quinolone resistance-determining region in the DNA gyrase gyrB gene of Escherichia coli. *Antimicrob. Agents Chemother.* **1991,** *35* (8) 1647-1650.

34. Takahashi, H.; Kikuchi, T.; Shoji, S.; Fujimura, S.; Lutfor, A. B.; Tokue, Y.; Nukiwa, T.; Watanabe, A., Characterization of gyrA, gyrB, grlA and grlB mutations in fluoroquinolone-resistant clinical isolates of Staphylococcus aureus. *J. Antimicrob. Chemother.* **1998,** *41* (1) 49-57.

35. Takei, M.; Fukuda, H.; Kishii, R.; Hosaka, M., Target Preference of 15 Quinolones against Staphylococcus aureus, Based on Antibacterial Activities and Target Inhibition. *Antimicrob. Agents Chemother.* **2001,** *45* (12) 3544-3547.

36. Pan, X.; Ambler, J.; Mehtar, S.; Fisher, L., Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in Streptococcus pneumoniae. *Antimicrob. Agents Chemother.* **1996,** *40* (10) 2321-2326.

37. Gootz, T.; Zaniewski, R.; Haskell, S.; Schmieder, B.; Tankovic, J.; Girard, D.; Courvalin, P.; Polzer, R., Activity of the new fluoroquinolone trovafloxacin (CP-99,219) against DNA gyrase and topoisomerase IV mutants of Streptococcus pneumoniae selected in vitro. *Antimicrob. Agents Chemother.* **1996,** *40* (12) 2691-2697.

38. Fukuda, H.; Hiramatsu, K., Primary Targets of Fluoroquinolones in Streptococcus pneumoniae. *Antimicrob. Agents Chemother.* **1999,** *43* (2) 410-412.

39. Morris, J. E.; Pan, X.-S.; Fisher, L. M., Grepafloxacin, a Dimethyl Derivative of Ciprofloxacin, Acts Preferentially through Gyrase in Streptococcus pneumoniae: Role of the C-5 Group in Target Specificity. *Antimicrob. Agents Chemother.* **2002,** *46* (2) 582-585.

40. Pan, X.-S.; Fisher, L. M., DNA Gyrase and Topoisomerase IV Are Dual Targets of Clinafloxacin Action in Streptococcus pneumoniae. *Antimicrob. Agents Chemother.* **1998,** *42* (11) 2810-2816.

41. Lynch, A. S., Efflux systems in bacterial pathogens: An opportunity for therapeutic intervention? An industry view. *Biochem. Pharmacol.* **2006,** *71* (7) 949-956.

42. Ubukata, K.; Itoh-Yamashita, N.; Konno, M., Cloning and expression of the norA gene for fluoroquinolone resistance in Staphylococcus aureus. *Antimicrob. Agents Chemother.* **1989,** *33* (9) 1535-1539.

43. (a) Schmitz, F. J.; Fluit, A. C.; Lückefahr, M.; Engler, B.; Hofmann, B.; Verhoef, J.; Heinz, H. P.; Hadding, U.; Jones, M. E., The effect of reserpine, an inhibitor of multidrug

efflux pumps, on the in-vitro activities of ciprofloxacin, sparfloxacin and moxifloxacin against clinical isolates of Staphylococcus aureus. *J. Antimicrob. Chemother.* **1998,** *42* (6) 807-810; (b) Beyer, R.; Pestova, E.; Millichap, J. J.; Stosor, V.; Noskin, G. A.; Peterson, L. R., A Convenient Assay for Estimating the Possible Involvement of Efflux of Fluoroquinolones by Streptococcus pneumoniae and Staphylococcus aureus: Evidence for Diminished Moxifloxacin, Sparfloxacin, and Trovafloxacin Efflux. *Antimicrob. Agents Chemother.* **2000,** *44* (3) 798-801.

44. Tran, J. H.; Jacoby, G. A., Mechanism of plasmid-mediated quinolone resistance. *Proc. Natl. Acad. Sci. U. S. A.* **2002,** *99* (8) 5638-5642.

45. (a) Nikaido, H.; Thanassi, D. G., Penetration of lipophilic agents with multiple protonation sites into bacterial cells: tetracyclines and fluoroquinolones as examples. *Antimicrob. Agents Chemother.* **1993,** *37* (7) 1393-1399; (b) Fresta, M.; Guccione, S.; Beccari, A. R.; Furneri, P. M.; Puglisi, G., Combining molecular modeling with experimental methodologies: mechanism of membrane permeation and accumulation of ofloxacin. *Bioorg. Med. Chem.* **2002,** *10* (12) 3871-3889.

46. (a) Piddock, L. J. V.; Jin, Y. F.; Griggs, D. J., Effect of hydrophobicity and molecular mass on the accumulation of fluoroquinolones by Staphylococcus aureus. *J. Antimicrob. Chemother.* **2001,** *47* (3) 261-270; (b) Asuquo, A. E.; Piddock, L. J. V., Accumulation and killing kinetics of fifteen quinolones for Escherichia coil, Staphylococcus aureus and Pseudomonas aeruginosa. *J. Antimicrob. Chemother.* **1993,** *31* (6) 865-880; (c) Furet, Y. X.; Deshusses, J.; Pechere, J. C., Transport of pefloxacin across the bacterial cytoplasmic membrane in quinolone-susceptible Staphylococcus aureus. *Antimicrob. Agents Chemother.* **1992,** *36* (11) 2506-2511; (d) McCaffrey, C.; Bertasso, A.; Pace, J.; Georgopapadakou, N. H., Quinolone accumulation in Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. *Antimicrob. Agents Chemother.* **1992,** *36* (8) 1601-1605.

47. Nikaido, H., Molecular Basis of Bacterial Outer Membrane Permeability Revisited. *Microbiol. Mol. Biol. Rev.* **2003,** *67* (4) 593-656.

48. (a) Mitscher, L. A.; Ma, Z., Structure-activity relationships of quinolones. In *Fluoroquinolone Antibiot.*, Ronald, A. R.; Low, D., Eds. Birkhaeuser Verlag: **2003**, 11-48; (b) Mitscher, L. A., Bacterial Topoisomerase Inhibitors: Quinolone and Pyridone Antibacterial Agents. *Chem. Rev.* **2005,** *105* (2) 559-592; (c) Peterson, L. R., Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity. *Clin. Infect. Dis.* **2001,** *33 Suppl 3* 180-186.

49. Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E.; Pernet, A. G., Synthesis and structure-activity relationships of novel arylfluoroquinolone antibacterial agents. *J. Med. Chem.* **1985,** *28* (11) 1558-1564.

50. (a) Hayakawa, I.; Atarashi, S.; Yokohama, S.; Imamura, M.; Sakano, K.; Furukawa, M., Synthesis and antibacterial activities of optically active ofloxacin. *Antimicrob. Agents Chemother.* **1986,** *29* (1) 163-164; (b) Hoshino, K.; Sato, K.; Akahane, K.; Yoshida, A.; Hayakawa, I.; Sato, M.; Une, T.; Osada, Y., Significance of the methyl group on the oxazine ring of ofloxacin derivatives in the inhibition of bacterial and mammalian type II topoisomerases. *Antimicrob. Agents Chemother.* **1991,** *35* (2) 309-312.

51. Yoshida, T.; Yamamoto, Y.; Orita, H.; Kakiuchi, M.; Takahashi, Y.; Itakura, M.; Kado, N.; Mitani, K.; Yasuda, S., Studies on quinolone antibacterials. IV. Structure-activity relationships of antibacterial activity and side effects for 5- or 8-substituted and 5,8-disubstituted-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids. *Chem. Pharm. Bull.* **1996,** *44* (5) 1074-1085.

52. Andersson, M. I.; MacGowan, A. P., Development of the quinolones. *J. Antimicrob. Chemother.* **2003,** *51* (suppl 1) 1-11.

53. Gray, J. L.; Almstead, J.-I. K.; Gallagher, C. P.; Hu, X. E.; Kim, N. K.; Taylor, C. J.; Twinem, T. L.; Wallace, C. D.; Ledoussal, B., Synthesis and biological testing of non-Fluorinated analogues of levofloxacin. *Bioorg. Med. Chem. Lett.* **2003,** *13* (14) 2373-2375.

54. Lawrence, L. E.; Wu, P.; Fan, L.; Gouveia, K. E.; Card, A.; Casperson, M.; Denbleyker, K.; Barrett, J. F., The inhibition and selectivity of bacterial topoisomerases by BMS-284756 and its analogues. *J. Antimicrob. Chemother.* **2001,** *48* (2) 195-201.

55. Sanchez, J. P.; Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Nichols, J. B.; Trehan, A. K., Quinolone antibacterial agents. Synthesis and structure-activity relationships of 8-substituted quinoline-3-carboxylic acids and 1,8-naphthyridine-3-carboxylic acids. *J. Med. Chem.* **1988,** *31* (5) 983-991.

56. (a) Bertino, J. J.; Fish, D., The safety profile of the fluoroquinolones. *Clin. Ther.* **2000,** *22* (7) 798-817; (b) Domagala, J. M., Structure-activity and structure-side-effect relationships for the quinolone antibacterials. *J. Antimicrob. Chemother.* **1994,** *33* (4) 685-706.

57. Fukuda, H.; Kishii, R.; Takei, M.; Hosaka, M., Contributions of the 8-Methoxy Group of Gatifloxacin to Resistance Selectivity, Target Preference, and Antibacterial Activity against Streptococcus pneumoniae. *Antimicrob. Agents Chemother.* **2001,** *45* (6) 1649-1653.

58. Dalhoff, A., Comparative In Vitro and In Vivo Activity of the C-8 Methoxy Quinolone Moxifloxacin and the C-8 Chlorine Quinolone BAY y 3118. *Clin. Infect. Dis.* **2001,** *32* (S1) 16-22.

59. Ball, P.; Stahlmann, R.; Kubin, R.; Choudhri, S.; Owens, R., Safety profile of oral and intravenous moxifloxacin: Cumulative data from clinical trials and postmarketing studies. *Clin. Ther.* **2004,** *26* (7) 940-950.

60. Cecchetti, V.; Fravolini, A.; Palumbo, M.; Sissi, C.; Tabarrini, O.; Terni, P.; Xin, T., Potent 6-Desfluoro-8-methylquinolones as New Lead Compounds in Antibacterial Chemotherapy1. *J. Med. Chem.* **1996,** *39* (25) 4952-4957.

61. Vázquez, J. L.; Merino, S.; Domenech, Ò.; Berlanga, M.; Viñas, M.; Montero, M. T.; Hernández-Borrell, J., Determination of the partition coefficients of a homologous series of ciprofloxacin: influence of the N-4 piperazinyl alkylation on the antimicrobial activity. *Int. J. Pharm.* **2001,** *220* (1-2) 53-62.

62. (a) Bermejo, M.; Merino, V.; Garrigues, T. M.; Delfina, J. M. P.; Mulet, A.; Vizet, P.; Trouiller, G.; Mercier, C., Validation of a Biophysical Drug Absorption Model by the PATQSAR System. *J. Pharm. Sci.* **1999,** *88* (4) 398-405; (b) Escribano, E.; Calpena, A.; Garrigues, T.; Freixas, J.; Domenech, J.; Moreno, J., Structure-absorption relationships of a series of 6-fluoroquinolones. *Antimicrob. Agents Chemother.* **1997,** *41* (9) 1996-2000.

63. Abuo-Rahma, G. E.-D. A. A.; Sarhan, H. A.; Gad, G. F. M., Design, synthesis, antibacterial activity and physicochemical parameters of novel N-4-piperazinyl derivatives of norfloxacin. *Bioorg. Med. Chem.* **2009,** *17* (11) 3879-3886.

64. (a) Alovero, F.; Nieto, M.; Mazzieri, M. R.; Then, R.; Manzo, R. H., Mode of Action of Sulfanilyl Fluoroquinolones. *Antimicrob. Agents Chemother.* **1998,** *42* (6) 1495-1498; (b) Nieto, M. J.; Alovero, F. d. L.; Manzo, R. H.; Mazzieri, M. R., A new class of fluoroquinolones: benzenesulfonamidefluoroquinolones (BSFQs), antibacterial activity and SAR studies. *Eur. J. Med. Chem.* **1999,** *34* (3) 209-214.

65. Alovero, F. L.; Pan, X.-S.; Morris, J. E.; Manzo, R. H.; Fisher, L. M., Engineering the Specificity of Antibacterial Fluoroquinolones: Benzenesulfonamide Modifications at C-7 of Ciprofloxacin Change Its Primary Target in Streptococcus pneumoniae from Topoisomerase IV to Gyrase. *Antimicrob. Agents Chemother.* **2000,** *44* (2) 320-325.

66. Foroumadi, A.; Mohammadhosseini, N.; Emami, S.; Letafat, B.; Faramarzi, M. A.; Samadi, N.; Shafiee, A., Synthesis and antibacterial activity of new 7-piperazinyl-quinolones containing a functionalized 2-(furan-3-yl)ethyl moiety. *Arch. Pharm. (Weinheim, Ger.)* **2007,** *340* (1) 47-52.

67. Letafat, B.; Emami, S.; Mohammadhosseini, N.; Faramarzi, M. A.; Samadi, N.; Shafiee, A.; Foroumadi, A., Synthesis and antibacterial activity of new N-[2-(thiophen-3-yl)ethyl] piperazinyl quinolones. *Chem. Pharm. Bull.* **2007,** *55* (6) 894-898.

68. Foroumadi, A.; Ghodsi, S.; Emami, S.; Najjari, S.; Samadi, N.; Faramarzi, M. A.; Beikmohammadi, L.; Shirazi, F. H.; Shafiee, A., Synthesis and antibacterial activity of new fluoroquinolones containing a substituted N-(phenethyl)piperazine moiety. *Bioorg. Med. Chem. Lett.* **2006,** *16* (13) 3499-3503.

69. Emami, S.; Foroumadi, A.; Samadi, N.; Faramarzi, M. A.; Rajabalian, S., Conformationally Constrained Analogs of N-Substituted Piperazinylquinolones: Synthesis and Antibacterial Activity of N-(2,3-Dihydro-4-hydroxyimino-4H-1-benzopyran-3-yl)-piperazinylquinolones. *Arch. Pharm. (Weinheim, Ger.)* **2009,** *342* (7) 405-411.

70. Emami, S.; Foroumadi, A.; Faramarzi, M. A.; Samadi, N., Synthesis and antibacterial activity of quinolone-based compounds containing a coumarin moiety. *Arch. Pharm. (Weinheim, Ger.)* **2008,** *341* (1) 42-48.

71. Shen, L. L.; Mitscher, L. A.; Sharma, P. N.; O'Donnell, T. J.; Chu, D. W. T.; Cooper, C. S.; Rosen, T.; Pernet, A. G., Mechanism of inhibition of DNA gyrase by quinolone antibacterials: a cooperative drug-DNA binding model. *Biochemistry* **1989,** *28* (9) 3886-3894.

72. Kerns, R. J.; Rybak, M. J.; Cheung, C. M., Susceptibility studies of piperazinyl-cross-linked fluoroquinolone dimers against test strains of Gram-positive and Gram-negative bacteria. *Diagn. Microbiol. Infect. Dis.* **2006,** *54* (4) 305-310.

73. (a) Kerns, R. J.; Rybak, M. J.; Kaatz, G. W.; Vaka, F.; Cha, R.; Grucz, R. G.; Diwadkar, V. U.; Ward, T. D., Piperazinyl-linked fluoroquinolone dimers possessing potent antibacterial activity against drug-resistant strains of Staphylococcus aureus. *Bioorg. Med. Chem. Lett.* **2003,** *13* (10) 1745-1749; (b) Kerns, R. J.; Rybak, M. J.; Kaatz, G. W.; Vaka, F.; Cha, R.; Grucz, R. G.; Diwadkar, V. U., Structural features of piperazinyl-linked ciprofloxacin dimers required for activity against drug-resistant strains of Staphylococcus aureus. *Bioorg. Med. Chem. Lett.* **2003,** *13* (13) 2109-2112.

74. Zhao, X.; Quinn, B.; Kerns, R.; Drlica, K., Bactericidal activity and target preference of a piperazinyl-cross-linked ciprofloxacin dimer with Staphylococcus aureus and Escherichia coli. *J. Antimicrob. Chemother.* **2006,** *58* (6) 1283-1286.

75. Gould, K. A.; Pan, X. S.; Kerns, R. J.; Fisher, L. M., Ciprofloxacin Dimers Target Gyrase in Streptococcus pneumoniae. *Antimicrob. Agents Chemother.* **2004,** *48* (6) 2108-2115.

76. Chu, D. T.; Fernandes, P. B., Structure-activity relationships of the fluoroquinolones. *Antimicrob. Agents Chemother.* **1989,** *33* (2) 131-135.

77. Chu, D. T. Tricyclic quinoline and naphthyride antibacterials. *U.S. Patent 4767762*, **1988**.

78. Kohlbrenner, W. E.; Wideburg, N.; Weigl, D.; Saldivar, A.; Chu, D. T., Induction of calf thymus topoisomerase II-mediated DNA breakage by the antibacterial isothiazoloquinolones A-65281 and A-65282. *Antimicrob. Agents Chemother.* **1992,** *36* (1) 81-86.

79. Wiles, J. A.; Wang, Q.; Lucien, E.; Hashimoto, A.; Song, Y.; Cheng, J.; Marlor, C. W.; Ou, Y.; Podos, S. D.; Thanassi, J. A.; Thoma, C. L.; Deshpande, M.; Pucci, M. J.;

Bradbury, B. J., Isothiazoloquinolones containing functionalized aromatic hydrocarbons at the 7-position: Synthesis and in vitro activity of a series of potent antibacterial agents with diminished cytotoxicity in human cells. *Bioorg. Med. Chem. Lett.* **2006,** *16* (5) 1272-1276.

80. Wiles, J. A.; Song, Y.; Wang, Q.; Lucien, E.; Hashimoto, A.; Cheng, J.; Marlor, C. W.; Ou, Y.; Podos, S. D.; Thanassi, J. A.; Thoma, C. L.; Deshpande, M.; Pucci, M. J.; Bradbury, B. J., Biological evaluation of isothiazoloquinolones containing aromatic heterocycles at the 7-position: In vitro activity of a series of potent antibacterial agents that are effective against methicillin-resistant Staphylococcus aureus. *Bioorg. Med. Chem. Lett.* **2006,** *16* (5) 1277-1281.

81. Wang, Q.; Lucien, E.; Hashimoto, A.; Pais, G. C. G.; Nelson, D. M.; Song, Y.; Thanassi, J. A.; Marlor, C. W.; Thoma, C. L.; Cheng, J.; Podos, S. D.; Ou, Y.; Deshpande, M.; Pucci, M. J.; Buechter, D. D.; Bradbury, B. J.; Wiles, J. A., Isothiazoloquinolones with Enhanced Antistaphylococcal Activities against Multidrug-Resistant Strains: Effects of Structural Modifications at the 6-, 7-, and 8-Positions. *J. Med. Chem.* **2007,** *50* (2) 199-210.

82. Pucci, M. J.; Cheng, J.; Podos, S. D.; Thoma, C. L.; Thanassi, J. A.; Buechter, D. D.; Mushtaq, G.; Vigliotti, G. A., Jr.; Bradbury, B. J.; Deshpande, M., In Vitro and In Vivo Antibacterial Activities of Heteroaryl Isothiazolones against Resistant Gram-Positive Pathogens. *Antimicrob. Agents Chemother.* **2007,** *51* (4) 1259-1267.

83. Cheng, J.; Thanassi, J. A.; Thoma, C. L.; Bradbury, B. J.; Deshpande, M.; Pucci, M. J., Dual Targeting of DNA Gyrase and Topoisomerase IV: Target Interactions of Heteroaryl Isothiazolones in Staphylococcus aureus. *Antimicrob. Agents Chemother.* **2007,** *51* (7) 2445-2453.

84. Patel, N. B.; Patel, A. L.; Chauhan, H. I., Synthesis of amide derivatives of quinolone and their antimicrobial studies. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2007,** *46B* (1) 126-134.

85. Sultana, N.; Arayne, M. S.; Rizvi, S. B. S.; Mesaik, M. A., Synthesis, characterization and biological evaluation of a series of levofloxacin carboxamide analogues. *Bull. Korean Chem. Soc.* **2009,** *30* (10) 2294-2298.

86. (a) Rivers, E. C.; Mancera, R. L., New anti-tuberculosis drugs in clinical trials with novel mechanisms of action. *Drug Discovery Today* **2008,** *13* (23-24) 1090-1098; (b) WHO, Global tuberculosis control: epidemiology, strategy, financing **2009**. http://www.who.int/tb/publications/global_report/2009/en/index.html

87. Johnson, R.; Streicher, E. M.; Louw, G. E.; Warren, R. M.; van Helden, P. D.; Victor, T. C., Drug resistance in Mycobacterium tuberculosis. *Curr. Issues Mol. Biol.* **2006,** *8* (2) 97-111.

88. Lowther, J.; Bryskier, A., Fluoroquinolones and tuberculosis. *Expert Opin. Invest. Drugs* **2002,** *11* (2) 233-258.

89. Loddenkemper, R.; Hauer, B., Drug-resistant tuberculosis: a worldwide epidemic poses a new challenge. *Dtsch. Arztebl. Int.* **2010,** *107* (1-2) 10-9.

90. WHO, Treatment of Tuberculosis: guidelines for national programmes **2010**. http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf.

91. Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M. A.; Rajandream, M. A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Taylor, K.; Whitehead, S.; Barrell, B. G., Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature* **1998,** *393* (6685) 537-544.

92. Aubry, A.; Mark Fisher, L.; Jarlier, V.; Cambau, E., First functional characterization of a singly expressed bacterial type II topoisomerase: The enzyme from Mycobacterium tuberculosis. *Biochem. Biophys. Res. Commun.* **2006,** *348* (1) 158-165.

93. Chatterjee, D., The mycobacterial cell wall: structure, biosynthesis and sites of drug action. *Curr. Opin. Chem. Biol.* **1997,** *1* (4) 579-588.

94. Jarlier, V.; Nikaido, H., Mycobacterial cell wall: Structure and role in natural resistance to antibiotics. *FEMS Microbiol. Lett.* **1994,** *123* (1-2) 11-18.

95. Daffé, M.; Etienne, G., The capsule of Mycobacterium tuberculosis and its implications for pathogenicity. *Tubercle and Lung Disease* **1999,** *79* (3) 153-169.

96. Almeida, D.; Nuermberger, E.; Tyagi, S.; Bishai, W. R.; Grosset, J., In Vivo Validation of the Mutant Selection Window Hypothesis with Moxifloxacin in a Murine Model of Tuberculosis. *Antimicrob. Agents Chemother.* **2007,** *51* (12) 4261-4266.

97. (a) Kocagoz, T.; Hackbarth, C.; Unsal, I.; Rosenberg, E.; Nikaido, H.; Chambers, H., Gyrase mutations in laboratory-selected, fluoroquinolone-resistant mutants of Mycobacterium tuberculosis H37Ra. *Antimicrob. Agents Chemother.* **1996,** *40* (8) 1768-1774; (b) Takiff, H. E.; Salazar, L.; Guerrero, C.; Philipp, W.; Huang, W. M.; Kreiswirth, B.; Cole, S. T.; Jacobs, W. R., Jr; Telenti, A., Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB genes and detection of quinolone resistance mutations. *Antimicrob. Agents Chemother.* **1994,** *38* (4) 773-780; (c) Cheng, A. F. B.; Yew, W. W.; Chan, E. W. C.; Chin, M. L.; Hui, M. M. M.; Chan, R. C. Y., Multiplex PCR Amplimer Conformation Analysis

for Rapid Detection of gyrA Mutations in Fluoroquinolone-Resistant Mycobacterium tuberculosis Clinical Isolates. *Antimicrob. Agents Chemother.* **2004,** *48* (2) 596-601.

98. Poole, K., Efflux-Mediated Resistance to Fluoroquinolones in Gram-Positive Bacteria and the Mycobacteria. *Antimicrob. Agents Chemother.* **2000,** *44* (10) 2595-2599.

99. Banerjee, S. K.; Bhatt, K.; Rana, S.; Misra, P.; Chakraborti, P. K., Involvement of an Efflux System in Mediating High Level of Fluoroquinolone Resistance inMycobacterium smegmatis. *Biochem. Biophys. Res. Commun.* **1996,** *226* (2) 362-368.

100. (a) Takiff, H. E.; Cimino, M.; Musso, M. C.; Weisbrod, T.; Martinez, R.; Delgado, M. B.; Salazar, L.; Bloom, B. R.; Jacobs, W. R., Efflux pump of the proton antiporter family confers low-level fluoroquinolone resistance in Mycobacterium smegmatis. *Proc. Natl. Acad. Sci. U. S. A.* **1996,** *93* (1) 362-366; (b) Liu, J.; Takiff, H.; Nikaido, H., Active efflux of fluoroquinolones in Mycobacterium smegmatis mediated by LfrA, a multidrug efflux pump. *J. Bacteriol.* **1996,** *178* (13) 3791-3795.

101. Montero, C.; Mateu, G.; Rodriguez, R.; Takiff, H., Intrinsic Resistance of Mycobacteriumsmegmatis to Fluoroquinolones May Be Influenced by New Pentapeptide Protein MfpA. *Antimicrob. Agents Chemother.* **2001,** *45* (12) 3387-3392.

102. Hegde, S. S.; Vetting, M. W.; Roderick, S. L.; Mitchenall, L. A.; Maxwell, A.; Takiff, H. E.; Blanchard, J. S., A Fluoroquinolone Resistance Protein from Mycobacterium tuberculosis That Mimics DNA. *Science* **2005,** *308* (5727) 1480-1483.

103. (a) Renau, T. E.; Sanchez, J. P.; Shapiro, M. A.; Dever, J. A.; Gracheck, S. J.; Domagala, J. M., Effect of Lipophilicity at N-1 on Activity of Fluoroquinolones against Mycobacteria. *J. Med. Chem.* **1995,** *38* (15) 2974-2977; (b) Renau, T. E.; Sanchez, J. P.; Gage, J. W.; Dever, J. A.; Shapiro, M. A.; Gracheck, S. J.; Domagala, J. M., 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) 729-735.

104. Lu, T.; Zhao, X.; Li, X.; Drlica-Wagner, A.; Wang, J. Y.; Domagala, J.; Drlica, K., Enhancement of Fluoroquinolone Activity by C-8 Halogen and Methoxy Moieties: Action against a Gyrase Resistance Mutant of Mycobacterium smegmatis and a Gyrase-Topoisomerase IV Double Mutant of Staphylococcus aureus. *Antimicrob. Agents Chemother.* **2001,** *45* (10) 2703-2709.

105. Dong, Y.; Xu, C.; Zhao, X.; Domagala, J.; Drlica, K., Fluoroquinolone Action against Mycobacteria: Effects of C-8 Substituents on Growth, Survival, and Resistance. *Antimicrob. Agents Chemother.* **1998,** *42* (11) 2978-2984.

106. Haemers, A.; Leysen, D. C.; Bollaert, W.; Zhang, M. Q.; Pattyn, S. R., Influence of N substitution on antimycobacterial activity of ciprofloxacin. *Antimicrob. Agents Chemother.* **1990,** *34* (3) 496-497.

107. Almeida, M. V.; Saraiva, M. F.; de Souza, M. V. N.; da Costa, C. F.; Vicente, F. R. C.; Lourenço, M. C. S., Synthesis and antitubercular activity of lipophilic moxifloxacin and gatifloxacin derivatives. *Bioorg. Med. Chem. Lett.* **2007,** *17* (20) 5661-5664.

108. (a) Sriram, D.; Yogeeswari, P.; Basha, J. S.; Radha, D. R.; Nagaraja, V., Synthesis and antimycobacterial evaluation of various 7-substituted ciprofloxacin derivatives. *Bioorg. Med. Chem.* **2005,** *13* (20) 5774-5778; (b) Sriram, D.; Bal, T. R.; Yogeeswari, P.; Radha, D. R.; Nagaraja, V., Evaluation of antimycobacterial and DNA gyrase inhibition of fluoroquinolone derivatives. *J. Gen. Appl. Microbiol.* **2006,** *52* (4) 195-200.

109. Senthilkumar, P.; Dinakaran, M.; Yogeeswari, P.; China, A.; Nagaraja, V.; Sriram, D., Antimycobacterial activities of novel fluoroquinolones. *Biomed. Pharmacother.* **2009,** *63* (1) 27-35.

110. Dinakaran, M.; Senthilkumar, P.; Yogeeswari, P.; China, A.; Nagaraja, V.; Sriram, D., Novel ofloxacin derivatives: Synthesis, antimycobacterial and toxicological evaluation. *Bioorg. Med. Chem. Lett.* **2008,** *18* (3) 1229-1236.

111. (a) Molina-Torres, C. A.; Ocampo-Candiani, J.; Rendon, A.; Pucci, M. J.; Vera-Cabrera, L., In Vitro Activity of a New Isothiazoloquinolone, ACH-702, against Mycobacterium tuberculosis and Other Mycobacteria. *Antimicrob. Agents Chemother.* **2010,** *54* (5) 2188-2190; (b) Pucci, M. J.; Ackerman, M.; Thanassi, J. A.; Shoen, C. M.; Cynamon, M. H., In Vitro Antituberculosis Activities of ACH-702, a Novel Isothiazoloquinolone, against Quinolone-Susceptible and Quinolone-Resistant Isolates. *Antimicrob. Agents Chemother.* **2010,** *54* (8) 3478-3480.

112. Drlica, K.; Zhao, X., DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol. Mol. Biol. Rev.* **1997,** *61* (3) 377-392.

113. Azarova, A. M.; Lyu, Y. L.; Lin, C.-P.; Tsai, Y.-C.; Lau, J. Y.-N.; Wang, J. C.; Liu, L. F., Roles of DNA topoisomerase II isozymes in chemotherapy and secondary malignancies. *Proc. Natl. Acad. Sci. U. S. A.* **2007,** *104* (26) 11014-11019.

114. (a) Hussy, P.; Maass, G.; Tummler, B.; Grosse, F.; Schomburg, U., Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase alpha primase complex, topoisomerases I and II, and growth of mammalian lymphoblasts. *Antimicrob. Agents Chemother.* **1986,** *29* (6) 1073-1078; (b) Hoshino, K.; Sato, K.; Une, T.; Osada, Y., Inhibitory effects of quinolones on DNA gyrase of Escherichia coli and topoisomerase II of fetal calf thymus. *Antimicrob. Agents Chemother.* **1989,** *33* (10) 1816-1818.

115. Barrett, J. F.; Gootz, T. D.; McGuirk, P. R.; Farrell, C. A.; Sokolowski, S. A., Use of in vitro topoisomerase II assays for studying quinolone antibacterial agents. *Antimicrob. Agents Chemother.* **1989,** *33* (10) 1697-1703.

116. (a) Robinson, M. J.; Martin, B. A.; Gootz, T. D.; McGuirk, P. R.; Moynihan, M.; Sutcliffe, J. A.; Osheroff, N., Effects of quinolone derivatives on eukaryotic topoisomerase II. A novel mechanism for enhancement of enzyme-mediated DNA cleavage. *J. Biol. Chem.* **1991,** *266* (22) 14585-14592; (b) Duan, W.; Rangan, A.; Vankayalapati, H.; Kim, M. Y.; Zeng, Q.; Sun, D.; Han, H.; Fedoroff, O. Y.; Nishioka, D.; Rha, S. Y.; Izbicka, E.; Von, H. D. D.; Hurley, L. H., Design and synthesis of fluoroquinophenoxazines that interact with human telomeric G-quadruplexes and their biological effects. *Mol. Cancer Ther.* **2001,** *1* (2) 103-20.

117. Advani, R. H.; Hurwitz, H. I.; Gordon, M. S.; Ebbinghaus, S. W.; Mendelson, D. S.; Wakelee, H. A.; Hoch, U.; Silverman, J. A.; Havrilla, N. A.; Berman, C. J.; Fox, J. A.; Allen, R. S.; Adelman, D. C., Voreloxin, a First-in-Class Anticancer Quinolone Derivative, in Relapsed/Refractory Solid Tumors: A Report on Two Dosing Schedules. *Clin. Cancer Res.* **2010,** *16* (7) 2167-2175.

118. Quarfloxin in Patients With Low to Intermediate Grade Neuroendocrine Carcinoma, Phase II **2008**. http://clinicaltrials.gov/ct2/show/NCT00780663A.

119. Roca, J., The mechanisms of DNA topoisomerases. *Trends Biochem. Sci.* **1995,** *20* (4) 156-160.

120. Caron, P. R.; Wang, J. C., Appendix II: Alignment of Primary Sequences of DNA Topoisomerases. In *Adv. Pharmacol. (San Diego, CA, U. S.)*, Leroy, F. L., Ed. Academic Press: **1994**, Vol. 29, Part 2, 271-297.

121. Wasserman, R. A.; Austin, C. A.; Fisher, L. M.; Wang, J. C., Use of Yeast in the Study of Anticancer Drugs Targeting DNA Topoisomerases: Expression of a Functional Recombinant Human DNA Topoisomerase IIα in Yeast. *Cancer Res.* **1993,** *53* (15) 3591-3596.

122. Nitiss, J. L., DNA topoisomerase II and its growing repertoire of biological functions. *Nat. Rev. Cancer* **2009,** *9* (5) 327-337.

123. (a) Deweese, J. E.; Osheroff, N., The DNA cleavage reaction of topoisomerase II: wolf in sheep's clothing. *Nucleic Acids Res.* **2009,** *37* (3) 738-748; (b) Nitiss, J. L., Targeting DNA topoisomerase II in cancer chemotherapy. *Nat. Rev. Cancer* **2009,** *9* (5) 338-350.

124. Larsen, A. K.; Escargueil, A. E.; Skladanowski, A., Catalytic topoisomerase II inhibitors in cancer therapy. *Pharmacol. Ther.* **2003,** *99* (2) 167-181.

125. Heisig, P., Type II topoisomerases—inhibitors, repair mechanisms and mutations. *Mutagenesis* **2009,** *24* (6) 465-469.

126. (a) Bromberg, K. D.; Burgin, A. B.; Osheroff, N., Quinolone Action against Human Topoisomerase IIα: Stimulation of Enzyme-Mediated Double-Stranded DNA Cleavage. *Biochemistry* **2003,** *42* (12) 3393-3398; (b) Elsea, S. H.; Osheroff, N.; Nitiss, J. L., Cytotoxicity of quinolones toward eukaryotic cells. Identification of topoisomerase II as the primary cellular target for the quinolone CP-115,953 in yeast. *J. Biol. Chem.* **1992,** *267* (19) 13150-13153.

127. Coughlin, S. A.; Danz, D. W.; Robinson, R. G.; Klingbeil, K. M.; Wentland, M. P.; Corbett, T. H.; Waud, W. R.; Zwelling, L. A.; Altschuler, E.; Bales, E.; Rake, J. B., Mechanism of action and antitumor activity of (S)-10-(2,6-dimethyl-4-pyridinyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyridol [1,2,3-de]-[1,4]benzothiazine-6-carboxylic acid (WIN 58161). *Biochem. Pharmacol.* **1995,** *50* (1) 111-122.

128. Hawtin, R. E.; Stockett, D. E.; Byl, J. A. W.; McDowell, R. S.; Tan, N.; Arkin, M. R.; Conroy, A.; Yang, W.; Osheroff, N.; Fox, J. A., Voreloxin Is an Anticancer Quinolone Derivative that Intercalates DNA and Poisons Topoisomerase II. *PLoS ONE* **2010,** *5* (4) e10186.

129. Permana, P. A.; Snapka, R. M.; Shen, L. L.; Chu, D. T. W.; Clement, J. J.; Plattner, J. J., Quinobenoxazines: A Class of Novel Antitumor Quinolones and Potent Mammalian DNA Topoisomerase II Catalytic Inhibitors. *Biochemistry* **1994,** *33* (37) 11333-11339.

130. Pommier, Y., DNA Topoisomerase I Inhibitors: Chemistry, Biology, and Interfacial Inhibition. *Chem. Rev.* **2009,** *109* (7) 2894-2902.

131. D'Arpa, P.; Beardmore, C.; Liu, L. F., Involvement of Nucleic Acid Synthesis in Cell Killing Mechanisms of Topoisomerase Poisons. *Cancer Res.* **1990,** *50* (21) 6919-6924.

132. You, Q.-D.; Li, Z.-Y.; Huang, C.-H.; Yang, Q.; Wang, X.-J.; Guo, Q.-L.; Chen, X.-G.; He, X.-G.; Li, T.-K.; Chern, J.-W., Discovery of a Novel Series of Quinolone and Naphthyridine Derivatives as Potential Topoisomerase I Inhibitors by Scaffold Modification. *J. Med. Chem.* **2009,** *52* (18) 5649-5661.

133. Ou, T.-m.; Lu, Y.-j.; Tan, J.-h.; Huang, Z.-s.; Wong, K.-Y.; Gu, L.-q., G-Quadruplexes: Targets in Anticancer Drug Design. *ChemMedChem.* **2008,** *3* (5) 690-713.

134. Kim, M.-Y.; Duan, W.; Gleason-Guzman, M.; Hurley, L. H., Design, Synthesis, and Biological Evaluation of a Series of Fluoroquinoanthroxazines with Contrasting Dual Mechanisms of Action against Topoisomerase II and G-Quadruplexes. *J. Med. Chem.* **2003,** *46* (4) 571-583.

135. Drygin, D.; Siddiqui-Jain, A.; O'Brien, S.; Schwaebe, M.; Lin, A.; Bliesath, J.; Ho, C. B.; Proffitt, C.; Trent, K.; Whitten, J. P.; Lim, J. K. C.; Von Hoff, D.; Anderes, K.; Rice, W. G., Anticancer Activity of CX-3543: A Direct Inhibitor of rRNA Biogenesis. *Cancer Res.* **2009,** *69* (19) 7653-7661.

136. Morris, P. G.; Fornier, M. N., Microtubule Active Agents: Beyond the Taxane Frontier. *Clin. Cancer Res.* **2008,** *14* (22) 7167-7172.

137. (a) Risinger, A. L.; Giles, F. J.; Mooberry, S. L., Microtubule dynamics as a target in oncology. *Cancer Treat. Rev.* **2009,** *35* (3) 255-261; (b) Bhalla, K. N., Microtubule-targeted anticancer agents and apoptosis. *Oncogene* **2003,** *22* (56) 9075-9086; (c) Cortes, J.; Baselga, J., Targeting the Microtubules in Breast Cancer Beyond Taxanes: The Epothilones. *Oncologist* **2007,** *12* (3) 271-280.

138. Rai, S. S.; Wolff, J., Localization of the Vinblastine-binding Site on β-Tubulin. *J. Biol. Chem.* **1996,** *271* (25) 14707-14711.

139. Li, L.; Wang, H.-K.; Kuo, S.-C.; Wu, T.-S.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K.-H., Antitumor Agents 155. Synthesis and Biological Evaluation of 3',6,7-Substituted 2-Phenyl-4-quinolones as Antimicrotubule Agents. *J. Med. Chem.* **1994,** *37* (20) 3400-3407.

140. Li, L.; Wang, H.-K.; Kuo, S.-C.; Wu, T.-S.; Lednicer, D.; Lin, C. M.; Hamel, E.; Lee, K.-H., Antitumor Agents. 150. 2',3',4',5',5,6,7-Substituted 2-Phenyl-4-quinolones and Related Compounds: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization. *J. Med. Chem.* **1994,** *37* (8) 1126-1135.

141. Lai, Y.-Y.; Huang, L.-J.; Lee, K.-H.; Xiao, Z.; Bastow, K. F.; Yamori, T.; Kuo, S.-C., Synthesis and biological relationships of 3',6-substituted 2-phenyl-4-quinolone-3-carboxylic acid derivatives as antimitotic agents. *Bioorg. Med. Chem.* **2005,** *13* (1) 265-275.

142. Chen, Y.-C.; Lu, P.-H.; Pan, S.-L.; Teng, C.-M.; Kuo, S.-C.; Lin, T.-P.; Ho, Y.-F.; Huang, Y.-C.; Guh, J.-H., Quinolone analogue inhibits tubulin polymerization and induces apoptosis via Cdk1-involved signaling pathways. *Biochem. Pharmacol.* **2007,** *74* (1) 10-19.

143. Elsea, S. H.; Westergaard, M.; Burden, D. A.; Lomenick, J. P.; Osheroff, N., Quinolones Share a Common Interaction Domain on Topoisomerase II with Other DNA Cleavage-Enhancing Antineoplastic Drugs. *Biochemistry* **1997,** *36* (10) 2919-2924.

144. Elsea, S. H.; McGuirk, P. R.; Gootz, T. D.; Moynihan, M.; Osheroff, N., Drug features that contribute to the activity of quinolones against mammalian topoisomerase II and cultured cells: correlation between enhancement of enzyme-mediated DNA cleavage in vitro and cytotoxic potential. *Antimicrob. Agents Chemother.* **1993,** *37* (10) 2179-2186.

145. Wentland, M. P.; Lesher, G. Y.; Reuman, M.; Gruett, M. D.; Singh, B.; Aldous, S. C.; Dorff, P. H.; Rake, J. B.; Coughlin, S. A., 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) 2801-2809.

146. (a) Tomita, K.; Tsuzuki, Y.; Shibamori, K.-i.; Tashima, M.; Kajikawa, F.; Sato, Y.; Kashimoto, S.; Chiba, K.; Hino, K., Synthesis and Structure−Activity Relationships of Novel

7-Substituted 1,4-Dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acids as Antitumor Agents. Part 1. *J. Med. Chem.* **2002,** *45* (25) 5564-5575; (b) Tsuzuki, Y.; Tomita, K.; Shibamori, K.-i.; Sato, Y.; Kashimoto, S.; Chiba, K., Synthesis and Structure−Activity Relationships of Novel 7-Substituted 1,4-Dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acids as Antitumor Agents. Part 2. *J. Med. Chem.* **2004,** *47* (8) 2097-2109.

147. Eissenstat, M. A.; Kuo, G.-H.; Weaver, J. D.; Wentland, M. P.; Robinson, R. G.; Klingbeil, K. M.; Danz, D. W.; Corbett, T. H.; Coughlin, S. A., 3-benzyl-quinolones: Novel, potent inhibitors of mammalian topoisomerase II. *Bioorg. Med. Chem. Lett.* **1995,** *5* (9) 1021-1026.

148. Wentland, M. P.; Aldous, S. C.; Gruett, M. D.; Perni, R. B.; Powles, R. G.; Danz, D. W.; Klingbeil, K. M.; Peverly, A. D.; Robinson, R. G.; Corbett, T. H.; Rake, J. B.; Coughlin, S. A., The antitumor activity of novel pyrazoloquinoline derivatives. *Bioorg. Med. Chem. Lett.* **1995,** *5* (4) 405-410.

149. Robinson, M. J.; Martin, B. A.; Gootz, T. D.; McGuirk, P. R.; Osheroff, N., Effects of novel fluoroquinolones on the catalytic activities of eukaryotic topoisomerase II: Influence of the C-8 fluorine group. *Antimicrob. Agents Chemother.* **1992,** *36* (4) 751-756.

150. Suto, M. J.; Domagala, J. M.; Roland, G. E.; Mailloux, G. B.; Cohen, M. A., Fluoroquinolones: relationships between structural variations, mammalian cell cytotoxicity and antimicrobial activity. *J. Med. Chem.* **1992,** *35* (25) 4745-4750.

151. Tsuzuki, Y.; Tomita, K.; Sato, Y.; Kashimoto, S.; Chiba, K., Synthesis and structure-activity relationships of 3-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridines as novel antitumor agents. *Bioorg. Med. Chem. Lett.* **2004,** *14* (12) 3189-3193.

152. Wentland, M. P.; Lesher, G. Y.; Reuman, M.; Pilling, G. M.; Saindane, M. T.; Perni, R. B.; Eissenstat, M. A.; Weaver Iii, J. D.; Singh, B.; Rake, J.; Coughlin, S. A., Relationship of structure of bridged (2,6-dimethyl-4-pyridinyl)quinolones to mammalian topoisomerase II inhibition. *Bioorg. Med. Chem. Lett.* **1993,** *3* (8) 1711-1716.

153. (a) Zeng, Q.; Kwok, Y.; Kerwin, S. M.; Mangold, G.; Hurley, L. H., Design of New Topoisomerase II Inhibitors Based upon a Quinobenzoxazine Self-Assembly Model. *J. Med. Chem.* **1998,** *41* (22) 4273-4278; (b) Kwok, Y.; Sun, D.; Clement, J. J.; Hurley, L. H., The quinobenzoxazines: relationship between DNA binding and biological activity. *Anti-Cancer Drug Des.* **1999,** *14* (5) 443-450.

154. Kwok, Y.; Zeng, Q.; Hurley, L. H., Structural Insight into a Quinolone-Topoisomerase II-DNA Complex. *J. Biol. Chem.* **1999,** *274* (24) 17226-17235.

155. (a) Weigt, M.; Wiese, M., A Comparative Molecular Field Analysis of Inhibitors of Tubulin Polymerization. *Quant. Struct.-Act. Relat.* **2000,** *19* (2) 142-148; (b) Zhang, S.-X.;

Feng, J.; Kuo, S.-C.; Brossi, A.; Hamel, E.; Tropsha, A.; Lee, K.-H., Antitumor Agents. 199. Three-Dimensional Quantitative Structure−Activity Relationship Study of the Colchicine Binding Site Ligands Using Comparative Molecular Field Analysis. *J. Med. Chem.* **2000,** *43* (2) 167-176.

156. Yamashita, Y.; Ashizawa, T.; Morimoto, M.; Hosomi, J.; Nakano, H., Antitumor Quinolones with Mammalian Topoisomerase II Mediated DNA Cleavage Activity. *Cancer Res.* **1992,** *52* (10) 2818-2822.

157. Fang, K.-C.; Chen, Y.-L.; Sheu, J.-Y.; Wang, T.-C.; Tzeng, C.-C., Synthesis, Antibacterial, and Cytotoxic Evaluation of Certain 7-Substituted Norfloxacin Derivatives. *J. Med. Chem.* **2000,** *43* (20) 3809-3812.

158. Foroumadi, A.; Emami, S.; Rajabalian, S.; Badinloo, M.; Mohammadhosseini, N.; Shafiee, A., N-Substituted piperazinyl quinolones as potential cytotoxic agents: Structure–activity relationships study. *Biomed. Pharmacother.* **2009,** *63* (3) 216-220.

159. Rajabalian, S.; Foroumadi, A.; Shafiee, A.; Emami, S., Functionalized N-(2-oxyiminoethyl)piperazinyl quinolones as new cytotoxic agents. *J. Pharm. Pharm. Sci.* **2007,** *10* (2) 153-158.

160. Holbeck, S. L.; Collins, J. M.; Doroshow, J. H., Analysis of Food and Drug Administration–Approved Anticancer Agents in the NCI60 Panel of Human Tumor Cell Lines. *Molecular Cancer Therapeutics* **2010,** *9* (5) 1451-1460.

161. Shaharyar, M.; Ali, M.; Abdullah, M., Synthesis and antiproliferative activity of 1-[(sub)]-6-fluoro-3-[(sub)]-1, 3,4-oxadiazol-2-yl-7-piperazino-1, 4-dihydro-4-quinolinone derivatives. *Med. Chem. Res.* **2007,** *16* (6) 292-299.

162. Hu, G. Q.; Zhang, Z. Q.; Xie, S. Q.; Huang, W. L., Synthesis and antitumor evaluation of C3/C3 fluoroquinolone dimers (I): Tethered with a fused heterocyclic s-triazolo[2,1-b][1,3,4]thiadiazole. *Chin. Chem. Lett.* **2010,** *21* (6) 661-663.

163. Sheehan, J. C.; Hess, G. P., A New Method of Forming Peptide Bonds. *J. Am. Chem. Soc.* **1955,** *77* (4) 1067-1068.

164. Sheehan, J.; Cruickshank, P.; Boshart, G., Notes- A Convenient Synthesis of Water-Soluble Carbodiimides. *J. Org. Chem.* **1961,** *26* (7) 2525-2528.

165. (a) Rebek, J.; Feitler, D., Improved method for the study of reaction intermediates. Mechanism of peptide synthesis mediated by carbodiimides. *J. Am. Chem. Soc.* **1973,** *95* (12) 4052-4053; (b) Rebek, J.; Feitler, D., Mechanism of the carbodiimide reaction. II. Peptide synthesis on the solid phase. *J. Am. Chem. Soc.* **1974,** *96* (5) 1606-1607.

166. Nudelman, A.; Levovich, I.; Cutts, S. M.; Phillips, D. R.; Rephaeli, A., The Role of Intracellularly Released Formaldehyde and Butyric Acid in the Anticancer Activity of Acyloxyalkyl Esters. *J. Med. Chem.* **2005,** *48* (4) 1042-1054.

167. Azéma, J.; Guidetti, B.; Malet-Martino, M.; Martino, R.; Roques, C., Efficient approach to acyloxymethyl esters of nalidixic acid and in vitro evaluation as intra-ocular prodrugs. *Bioorg. Med. Chem.* **2006,** *14* (8) 2569-2580.

168. (a) Binderup, E.; Hansen, E. T., Chlorosulfates as Reagents in the Synthesis of Carboxylic Acid Esters Under Phase-Transfer Conditions. *Synth. Commun.* **1984,** *14* (9) 857 - 864; (b) Harada, N.; Hongu, M.; Tanaka, T.; Kawaguchi, T.; Hashiyama, T.; Tsujihara, K., A Simple Preparation of Chloromethyl Esters of the Blocked Amino Acids. *Synth. Commun.* **1994,** *24* (6) 767 - 772.

169. Iyer, R. P.; Yu, D.; Ho, N.-h.; Agrawal, S., Synthesis of Iodoalkylacylates and Their Use in the preparation of S-Alkyl Phosphorothiolates. *Synth. Commun.* **1995,** *25* (18) 2739 - 2749.

170. Maeda, Y.; Omoda, K.; Konishi, T.; Takahashi, M.; Kihira, K.; Hibino, S.; Tsukiai, S., Effects of aluminium-containing antacid on bioavailability of ofloxacin following oral administration of pivaloyloxymethyl ester of ofloxacin as prodrug. *Biol. Pharm. Bull.* **1993,** *16* (6) 594-9.

171. (a) Bougrin, K.; Loupy, A.; Soufiaoui, M., Microwave-assisted solvent-free heterocyclic synthesis. *J. Photochem. Photobiol., C* **2005,** *6* (2-3) 139-167; (b) Arya, K.; Agarwal, M., Microwave prompted multigram synthesis, structural determination, and photo-antiproliferative activity of fluorinated 4-hydroxyquinolinones. *Bioorg. Med. Chem. Lett.* **2007,** *17* (1) 86-93; (c) Balamurugan, K.; Jeyachandran, V.; Perumal, S.; Manjashetty, T. H.; Yogeeswari, P.; Sriram, D., A microwave-assisted, facile, regioselective Friedländer synthesis and antitubercular evaluation of 2,9-diaryl-2,3-dihydrothieno-[3,2-b]quinolines. *Eur. J. Med. Chem.* **2010,** *45* (2) 682-688.

172. Ortmann, R.; Wiesner, J.; Reichenberg, A.; Henschker, D.; Beck, E.; Jomaa, H.; Schlitzer, M., Acyloxyalkyl ester prodrugs of FR900098 with improved in vivo anti-malarial activity. *Bioorg. Med. Chem. Lett.* **2003,** *13* (13) 2163-2166.

173. (a) Carpino, L. A., 1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive. *J. Am. Chem. Soc.* **1993,** *115* (10) 4397-4398; (b) Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M., The Uronium/Guanidinium Peptide Coupling Reagents: Finally the True Uronium Salts. *Angew. Chem., Int. Ed.* **2002,** *41* (3) 441-445.

174. Jang, D. O.; Park, D. J.; Kim, J., A mild and efficient procedure for the preparation of acid chlorides from carboxylic acids. *Tetrahedron Lett.* **1999,** *40* (29) 5323-5326.

175. Carpino, L. A.; El-Faham, A., Effect of Tertiary Bases on O-Benzotriazolyluronium Salt-Induced Peptide Segment Coupling. *J. Org. Chem.* **1994,** *59* (4) 695-698.

176. Carpino, L. A.; Ionescu, D.; El-Faham, A., Peptide Coupling in the Presence of Highly Hindered Tertiary Amines. *J. Org. Chem.* **1996,** *61* (7) 2460-2465.

177. Li, P.; Xu, J. C., The development of highly efficient onium-type peptide coupling reagents based upon rational molecular design. *J. Pept. Res.* **2001,** *58* (2) 129-139.

178. Carey, F. A.; Sundberg, R. J., *Advanced Organic Chemistry - Part B: Reactions and Synthesis (5th Edition)*. Springer - Verlag: **2007**.

179. (a) Chantarasriwong, O.; Jang, D. O.; Chavasiri, W., A practical and efficient method for the preparation of sulfonamides utilizing Cl3CCN/PPh3. *Tetrahedron Lett.* **2006,** *47* (42) 7489-7492; (b) Vágó, I.; Greiner, I., A useful acylation method using trichloroacetonitrile and triphenylphosphine for solid phase organic synthesis. *Tetrahedron Lett.* **2002,** *43* (34) 6039-6041.

180. (a) Di, L.; Kerns, E. H.; Hong, Y.; Chen, H., Development and application of high throughput plasma stability assay for drug discovery. *Int. J. Pharm.* **2005,** *297* (1-2) 110-119; (b) Hitchingham, L.; Thomas, V. H., Development of a semi-automated chemical stability system to analyze solution based formulations in support of discovery candidate selection. *J. Pharm. Biomed. Anal.* **2007,** *43* (2) 522-526; (c) Kibbey, C. E.; Poole, S. K.; Robinson, B.; Jackson, J. D.; Durham, D., An integrated process for measuring the physicochemical properties of drug candidates in a preclinical discovery environment. *J. Pharm. Sci.* **2001,** *90* (8) 1164-1175.

181. Ng, E. Y.; Trucksis, M.; Hooper, D. C., Quinolone resistance mediated by norA: physiologic characterization and relationship to flqB, a quinolone resistance locus on the Staphylococcus aureus chromosome. *Antimicrob. Agents Chemother.* **1994,** *38* (6) 1345-1355.

182. (a) Chapman, J. S.; Georgopapadakou, N. H., Routes of quinolone permeation in Escherichia coli. *Antimicrob. Agents Chemother.* **1988,** *32* (4) 438-442; (b) Marshall, A. J. H.; Piddock, L. J. V., Interaction of divalent cations, quinolones and bacteria. *J. Antimicrob. Chemother.* **1994,** *34* (4) 465-483.

183. (a) Jazayeri, S.; Moshafi, M. H.; Firoozpour, L.; Emami, S.; Rajabalian, S.; Haddad, M.; Pahlavanzadeh, F.; Esnaashari, M.; Shafiee, A.; Foroumadi, A., Synthesis and antibacterial activity of nitroaryl thiadiazole-gatifloxacin hybrids. *Eur. J. Med. Chem.* **2009,** *44* (3) 1205-1209; (b) Foroumadi, A.; Emami, S.; Mansouri, S.; Javidnia, A.; Saeid-Adeli, N.;

Shirazi, F. H.; Shafiee, A., Synthesis and antibacterial activity of levofloxacin derivatives with certain bulky residues on piperazine ring. *Eur. J. Med. Chem.* **2007,** *42* (7) 985-992.

184. Jefferson, E. A.; Swayze, E. E.; Osgood, S. A.; Miyaji, A.; Risen, L. M.; Blyn, L. B., Antibacterial activity of quinolone–Macrocycle conjugates. *Bioorg. Med. Chem. Lett.* **2003,** *13* (10) 1635-1638.

185. (a) Aubry, A.; Pan, X. S.; Fisher, L. M.; Jarlier, V.; Cambau, E., Mycobacterium tuberculosis DNA Gyrase: Interaction with Quinolones and Correlation with Antimycobacterial Drug Activity. *Antimicrob. Agents Chemother.* **2004,** *48* (4) 1281-1288; (b) Levofloxacin. *Tuberculosis* **2008,** *88* (2) 119-121; (c) Shandil, R. K.; Jayaram, R.; Kaur, P.; Gaonkar, S.; Suresh, B. L.; Mahesh, B. N.; Jayashree, R.; Nandi, V.; Bharath, S.; Balasubramanian, V., Moxifloxacin, Ofloxacin, Sparfloxacin, and Ciprofloxacin against Mycobacterium tuberculosis: Evaluation of In Vitro and Pharmacodynamic Indices That Best Predict In Vivo Efficacy. *Antimicrob. Agents Chemother.* **2007,** *51* (2) 576-582; (d) Abdel-Rahman, H. M.; El-Koussi, N. A.; Hassan, H. Y., Fluorinated 1,2,4-Triazolo[1,5-a]pyrimidine-6-carboxylic Acid Derivatives as Antimycobacterial Agents. *Arch. Pharm.* **2009,** *342* (2) 94-99; (e) Rodríguez, J. C.; Ruiz, M.; Climent, A.; Royo, G., In vitro activity of four fluoroquinolones against Mycobacterium tuberculosis. *Int. J. Antimicrob. Agents* **2001,** *17* (3) 229-231; (f) Vernon, A.; Burman, W., New treatment regimens for drug-sensitive tuberculosis: fluoroquinolones and enhanced rifamycins. *Respirology* **2008,** *13* S116-S124.

186. (a) Lamoral-Theys, D.; Andolfi, A.; Van Goietsenoven, G.; Cimmino, A.; Le Calvé, B.; Wauthoz, N.; Mégalizzi, V. r.; Gras, T.; Bruyère, C. l.; Dubois, J.; Mathieu, V. r.; Kornienko, A.; Kiss, R.; Evidente, A., Lycorine, the Main Phenanthridine Amaryllidaceae Alkaloid, Exhibits Significant Antitumor Activity in Cancer Cells That Display Resistance to Proapoptotic Stimuli: An Investigation of Structure−Activity Relationship and Mechanistic Insight. *J. Med. Chem.* **2009,** *52* (20) 6244-6256; (b) Lefranc, F.; Mijatovic, T.; Kondo, Y.; Sauvage, S.; Roland, I.; Debeir, O.; Krstic, D.; Vasic, V.; Gailly, P.; Kondo, S.; Blanco, G.; Kiss, R., Targeting the alpha 1 subunit of the sodium pump to combat glioblastoma cells. *Neurosurgery* **2008,** *62* (1) 211-221; (c) Ingrassia, L.; Lefranc, F.; Dewelle, J.; Pottier, L.; Mathieu, V. r.; Spiegl-Kreinecker, S.; Sauvage, S. b.; El Yazidi, M.; Dehoux, M. l.; Berger, W.; Van Quaquebeke, E.; Kiss, R., Structure−Activity Relationship Analysis of Novel Derivatives of Narciclasine (an Amaryllidaceae Isocarbostyril Derivative) as Potential Anticancer Agents. *J. Med. Chem.* **2009,** *52* (4) 1100-1114.

187. Mijatovic, T.; Mathieu, V.; Gaussin, J.-F.; De, N. N.; Ribaucour, F.; Van, Q. E.; Dumont, P.; Darro, F.; Kiss, R., Cardenolide-induced lysosomal membrane permeabilization

demonstrates therapeutic benefits in experimental human non-small cell lung cancers. *Neoplasia (Ann Arbor, MI, U. S.)* **2006,** *8* (5) 402-412.

188. Dumont, P.; Ingrassia, L.; Rouzeau, S.; Ribaucour, F.; Thomas, S.; Roland, I.; Darro, F.; Lefranc, F.; Kiss, R., The Amaryllidaceae isocarbostyril narciclasine induces apoptosis by activation of the death receptor and/or mitochondrial pathways in cancer cells but not in normal fibroblasts. *Neoplasia (Ann Arbor, MI, U. S.)* **2007,** *9* (9) 766-776.

189. Yao, Y.; Jia, X.-Y.; Tian, H.-Y.; Jiang, Y.-X.; Xu, G.-J.; Qian, Q.-J.; Zhao, F.-K., Comparative proteomic analysis of colon cancer cells in response to Oxaliplatin treatment. *Biochim. Biophys. Acta, Proteins Proteomics* **2009,** *1794* (10) 1433-1440.

190. (a) Delfourne, E.; Darro, F.; Portefaix, P.; Galaup, C.; Bayssade, S.; Bouteillé, A.; Le Corre, L.; Bastide, J.; Collignon, F.; Lesur, B.; Frydman, A.; Kiss, R., Synthesis and In Vitro Antitumor Activity of Novel Ring D Analogues of the Marine Pyridoacridine Ascididemin: Structure−Activity Relationship. *J. Med. Chem.* **2002,** *45* (17) 3765-3771; (b) Van Quaquebeke, E.; Simon, G.; André, A.; Dewelle, J.; Yazidi, M. E.; Bruyneel, F.; Tuti, J.; Nacoulma, O.; Guissou, P.; Decaestecker, C.; Braekman, J.-C.; Kiss, R.; Darro, F., Identification of a Novel Cardenolide (2‘‘-Oxovoruscharin) from Calotropis procera and the Hemisynthesis of Novel Derivatives Displaying Potent in Vitro Antitumor Activities and High in Vivo Tolerance: Structure−Activity Relationship Analyses. *J. Med. Chem.* **2005,** *48* (3) 849-856.

191. Darro, F.; Decaestecker, C.; Gaussin, J. F.; Mortier, S.; Van Ginckel, R.; Kiss, R., Are syngeneic mouse tumor models still valuable experimental models in the field of anti-cancer drug discovery? *Int. J. Oncol.* **2005,** *27* (3) 607-16.

192. Mondal, E. R.; Das, S. K.; Mukherjee, P., Comparative evaluation of antiproliferative activity and induction of apoptosis by some fluoroquinolones with a human non-small cell lung cancer cell line in culture. *Asian Pac. J. Cancer Prev.* **2004,** *5* (2) 196-204.

193. Yamakuchi, M.; Nakata, M.; Kawahara, K.-i.; Kitajima, I.; Maruyama, I., New quinolones, ofloxacin and levofloxacin, inhibit telomerase activity in transitional cell carcinoma cell lines. *Cancer Lett.* **1997,** *119* (2) 213-219.

194. Takei, M.; Fukuda, H.; Yasue, T.; Hosaka, M.; Oomori, Y., Inhibitory Activities of Gatifloxacin (AM-1155), a Newly Developed Fluoroquinolone, against Bacterial and Mammalian Type II Topoisomerases. *Antimicrob. Agents Chemother.* **1998,** *42* (10) 2678-2681.

Dérivés lipophiles de la ciprofloxacine et de la lévofloxacine : synthèse et évaluation de leurs activités antibactérienne, antimycobactérienne et antiprolifération

La ciprofloxacine (CIP) et la lévofloxacine (LEV) appartiennent à la large famille des antibiotiques de type fluoroquinolone (FQ). Ce sont des agents antibactériens qui ont également été recommandés pour le traitement de la tuberculose. Malgré tous leurs avantages, l'apparition des phénomènes de résistance dans les souches pathogènes bactériennes et *M. tuberculosis* devient un problème majeur. C'est pourquoi, le besoin de développer de nouveaux agents existe toujours.

Des études ont montré que CIP et LEV ont des activités antiproliférative et apoptotique contre plusieurs lignées de cellules cancéreuses. Le mécanisme exact de cette activité des FQs est inconnu, mais il a été prouvé qu'ils peuvent inhiber la topoisomérase II mammifère, les G-quadruplex, et la polymérisation de tubuline. De plus, nous avons mis en évidence que certains dérivés lipophiles de la CIP possèdent une activité antiproliférative. Le greffage de longues chaînes alkyles pourrait donc conduire à des agents anticancéreux.

Au cours de cette thèse, nous avons développé et synthétisé cinq séries des dérivés lipophiles de la CIP et de la LEV. Afin de contourner les difficultés synthétiques rencontrées (en particulier la faible réactivité de l'acide carboxylique en C-3 du motif quinolone) nous avons réalisé différents essais d'optimisation des conditions réactionnelles. L'influence de différents paramètres a été étudiée (des réactions ont été conduites sous microondes, en présence ou absence de catalyseur) ce qui nous a permis d'améliorer les rendements et de réduire le temps de réaction.

Les activités biologiques des cinq séries de dérivés ont été évaluées *in vitro* et pour les analogues les plus actifs les DMT ont été déterminées *in vivo*. L'analyse détaillée des activités antibactérienne, antimycobactérienne et antiproliférative nous a permis de mettre en évidence quelques règles générales de relation structure-activité concernant l'influence du type de substitution du noyau quinolone, de la longueur de la chaîne alkyle et de la structure type dimère ou monomère sur les différentes cibles biologiques.

Lipophilic ciprofloxacin and levofloxacin derivatives: synthesis and evaluation as antibiotic, antimycobacterial and antiproliferative agents

Ciprofloxacin (CIP) and levofloxacin (LEV) are members of the large family of fluoroquinolone (FQ) antibiotics. Though they have proved to be reliable and effective antibacterial broad-spectrum agents, with several members also approved as second-line drugs for tuberculosis treatment. Nevertheless, the emerging resistance among key bacterial pathogens and *M. tuberculosis* hinders their future effectiveness. So there is a constant need for new compounds which could help to overcome these obstacles.

In addition, studies have shown that LEV and CIP have antiproliferative and apoptotic activities against several cancer cell lines. The exact mechanism by which FQs exert their tumour growth inhibitory activity and lead to cell death is not fully understood but these molecules could inhibit mammalian topoisomerase II, G-quadruplexes and tubulin polymerization. Moreover, we reported that some 7-(4-substituted)piperazin-1-yl) derivatives of CIP with increased lipophilicity displayed increased antiproliferative activity *in vitro*, suggesting that grafting long alkyl chain could give rise to antitumor agents.

During the course of the thesis, we have designed and synthesized five series of CIP and LEV derivatives. A number of optimization runs were conducted in order to overcome the low reactivity of C-3 carboxylic acid on quinolone core. The influence of various parameters was examined (microwave-assisted synthesis versus thermal conditions, presence or absence of catalyst), which permitted us to increase the yields and decrease the reaction time.

Biological evaluation of these five series of FQ analogs was conducted in vitro together with in vivo MTD determination for the most potent ones. The detailed analysis of antimicrobial, antimycobacterial and antiproliferative activities permitted us to deduce general structure-activity relationships concerning the influence of substitution type on quinolone core, the length of the grafted alkyl chain and the "dimeric" or "monomeric" structure on the selectivity and potency against these various biological targets.