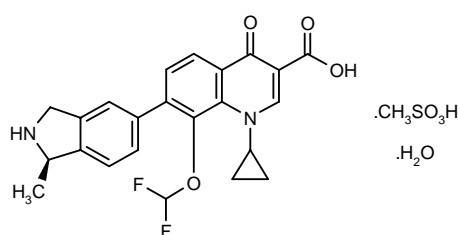


## T-3811ME

### Quinolone Antibacterial

BMS-284756

1-Cyclopropyl-8-(difluoromethoxy)-7-[1(*R*)-methyl-2,3-dihydro-1*H*-isoindol-5-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid monomethanesulfonate monohydrate



$C_{23}H_{20}F_2N_2O_4 \cdot CH_4O_3S \cdot H_2O$

Mol wt: 540.5374

CAS: 223652-90-2

CAS: 194804-75-6 (as anhydrous free base)

CAS: 223652-89-9 (as monosulfate)

CAS: 223652-87-7 (as monoacetate)

CAS: 223652-85-5 (as monosodium salt)

CAS: 223652-83-3 (as monophosphate)

CAS: 223652-82-2 (as monomethanesulfonate)

CAS: 223652-81-1 (as monohydrochloride)

CAS: 223652-88-8 (as magnesium salt)

CAS: 223652-86-6 [as citrate (1:1)]

EN: 254897

### Synthesis

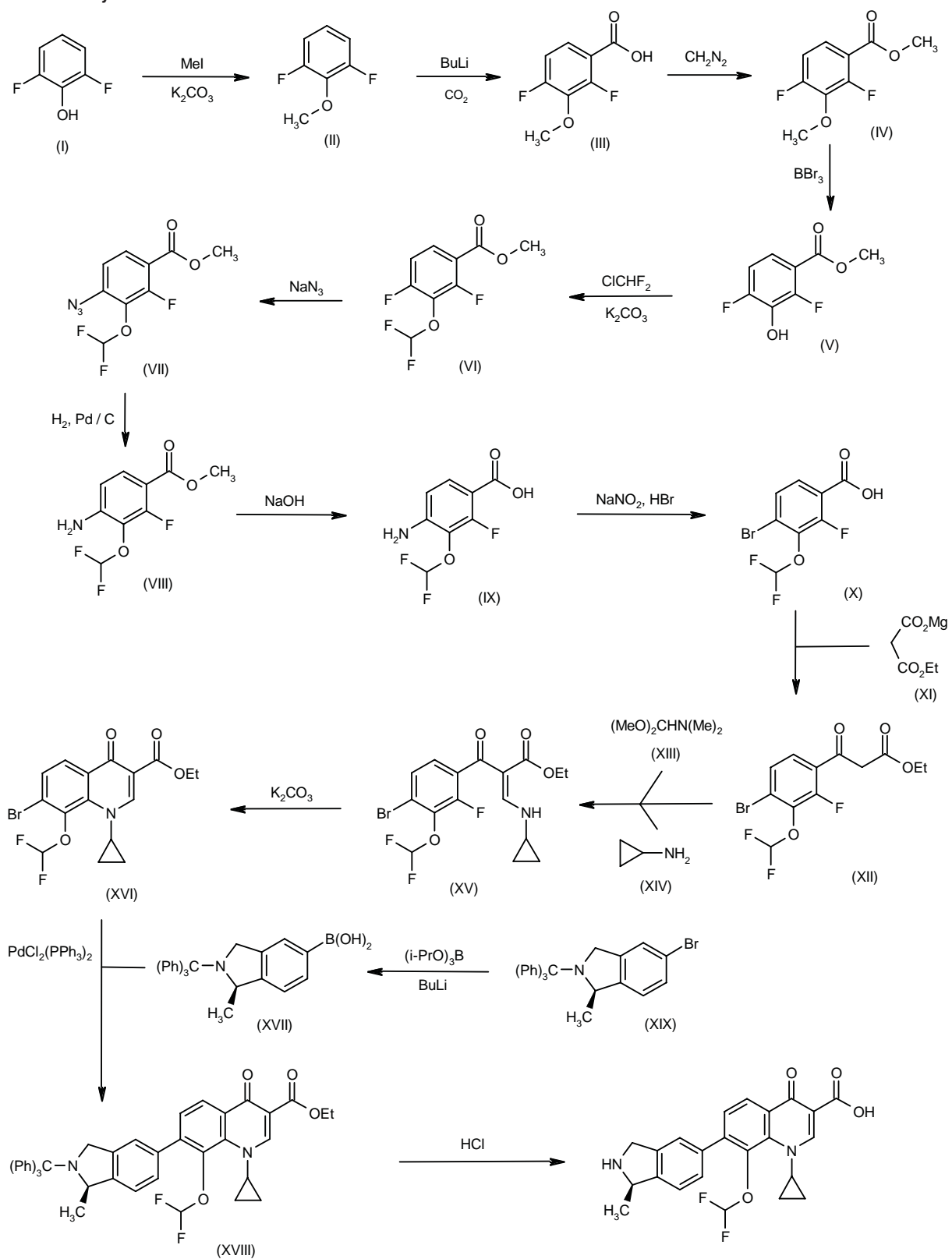
T-3811 has been obtained by two similar ways:

1) The methylation of 2,6-difluorophenol (I) with methyl iodide and  $K_2CO_3$  in DMF gives 2,6-difluoroanisole (II), which by treatment with butyl lithium and  $CO_2$  yields 2,4-difluoro-3-methoxybenzoic acid (III). The methylation of (III) with diazomethane in ether affords the methyl ester (IV), which by reaction with  $BBr_3$  in dichloromethane results in 2,4-difluoro-3-hydroxybenzoic acid methyl ester (V). The alkylation of (V) with chlorodifluoromethane and  $K_2CO_3$  in DMF gives 3-(difluoromethoxy)-2,4-difluorobenzoic acid methyl ester (VI), which is treated with sodium azide in DMSO, yielding the azido derivative (VII). The reduction of (VII) with  $H_2$  over Pd/C in ethanol affords 3-amino-2,4-difluorobenzoic acid methyl ester (VIII), which is hydrolyzed with NaOH in ethanol, giving the free acid (IX). The reaction of (IX) with

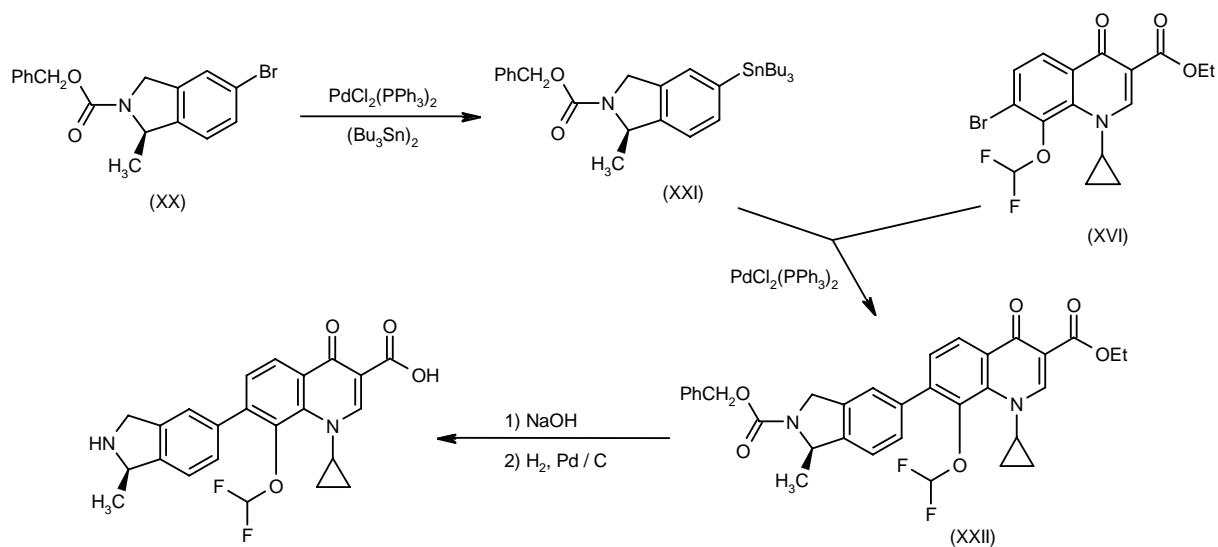
$NaNO_2$  and HBr yields 4-bromo-3-(difluoromethoxy)-2-fluorobenzoic acid (X), which is condensed with the magnesium salt of malonic acid monoethyl ester (XI) by means of CDI in THF, affording the 3-oxopropionate (XII). The reaction of (XII) with dimethylformamide dimethylacetal (XIII) and cyclopropylamine (XIV) by means of acetic anhydride in dichloromethane gives the 3-(cyclopropylamino)acrylate (XV), which is cyclized by means of  $K_2CO_3$  in hot DMSO, yielding quinolone (XVI). The condensation of (XVI) with the isoindolylboronic acid derivative (XVII) – obtained by reaction of 5-bromo-1(*R*)-methyl-2-tritylisoindoline (XIX) with triisopropyl borate and butyl lithium in THF – by means of bis(triphenylphosphine)palladium(II) chloride as catalyst in refluxing toluene affords the protected compound (XVIII), which is finally deprotected with HCl in ethanol (1). Scheme 1.

2) The reaction of 2-(benzyloxycarbonyl)-5-bromo-1(*R*)-methylisoindoline (XX) with hexabutylstannane and bis(triphenylphosphine)palladium(II) chloride in toluene gives the corresponding tributyltin derivative (XXI), which is condensed with the already described quinolone (XVI) by means of the palladium catalyst as before, yielding the protected compound (XXII), which is deprotected by hydrolysis of the ester group with NaOH, followed by hydrogenolysis with  $H_2$  over Pd/C in acetic acid (1, 2). Scheme 2.

The chiral indolines (XIX) and (XX) have been obtained as follows: The condensation of racemic 5-bromo-2-methylindoline (XXIII) with *N*-(benzyloxycarbonyl)-L-phenylalanine (XXIV) by means of DIC and triethylamine in THF gives the diastereomeric mixture of indolines (XXV), which is separated by column chromatography, yielding the pure enantiomer (XXVI). The cleavage of (XXVI) with 6N HCl affords 5-bromo-1(*R*)-methylisoindoline (XXVII), which is treated with trityl chloride and triethylamine in dichloromethane to give indoline (XIX), or with benzyl chloroformate and triethylamine to give indoline (XX) (1). Scheme 3.



Scheme 2: Synthesis of T-3811ME



Scheme 3: Synthesis of Intermediates (XIX) and (XX)

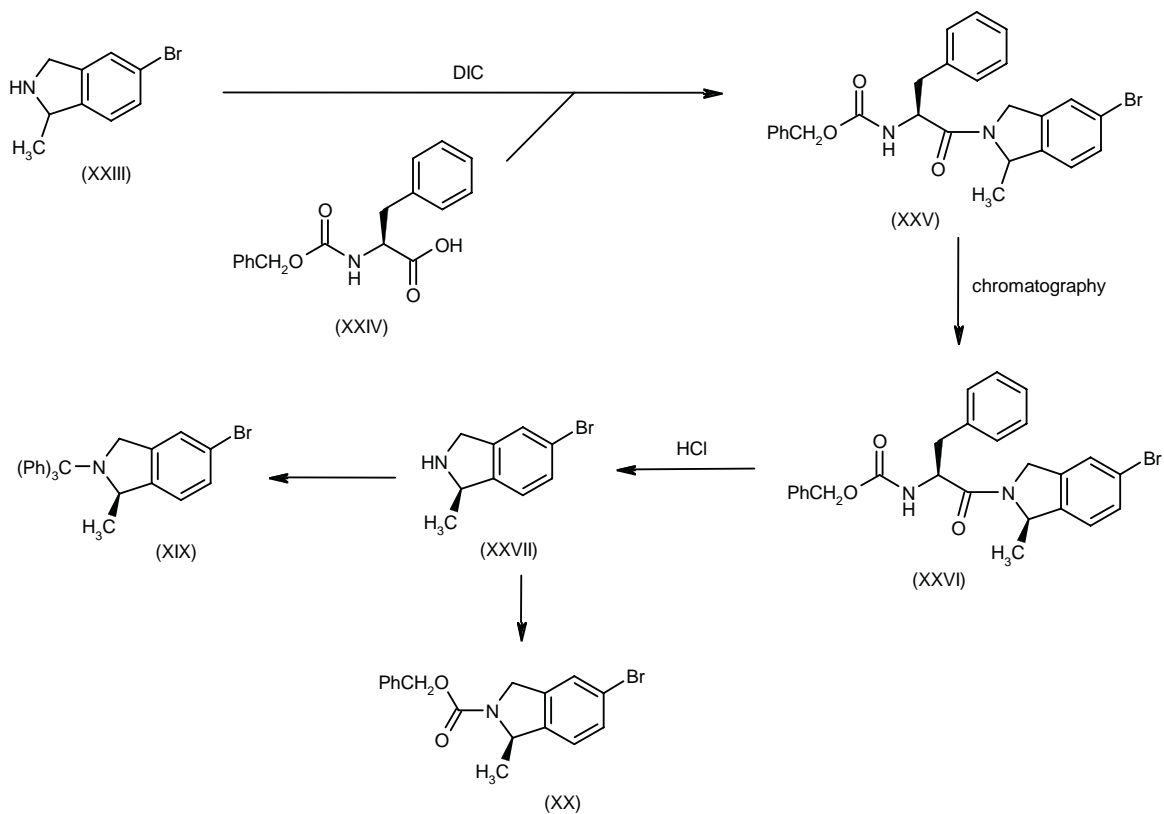
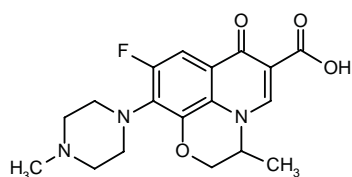
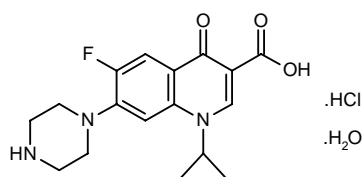


Table I: Chemical structures of marketed fluoroquinolone antibacterial agents (Prous Science Ensemble database).

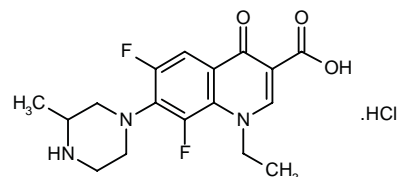
Generic name	Trade name/Company	Year Introduced
1. Ofloxacin	Tarivid (Daiichi Pharm.)	1985
2. Ciprofloxacin HCl	Cipro; Ciproxin (Bayer)	1986
3. Lomefloxacin HCl	Bareon (BASF); Maxaquin (Searle)	1989
4. Tosufloxacin tosilate <sup>1</sup>	Ozex (Toyama); Tosuxacin (Dainabot)	1990
5. Rufloxacin HCl	Qari (Mediolanum)	1992
6. Levofloxacin	Cravit (Daiichi Pharm.)	1993
7. Nadifloxacin <sup>2</sup>	Acuatim (Otsuka)	1993
8. Sparfloxacin	Spara (Dainippon); Zagam (Aventis)	1993
9. Grepafloxacin HCl <sup>3</sup>	Raxar; Vaxar (Otsuka; Glaxo Wellcome)	1997
10. Trovafloxacin mesilate <sup>1*</sup>	Trovan (Pfizer)	1998
11. Moxifloxacin HCl	Avelox; Avalox (Bayer)	1999



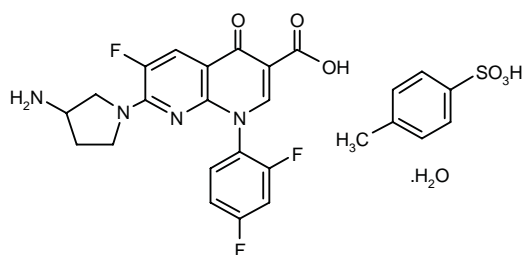
(1)



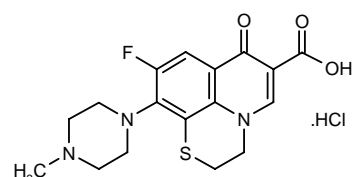
(2)



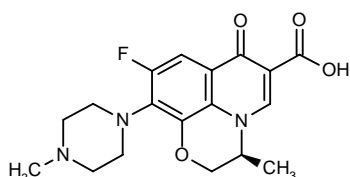
(3)



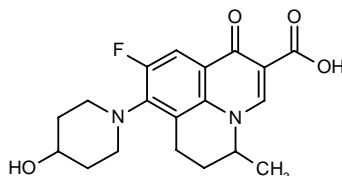
(4)



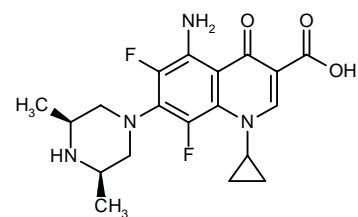
(5)



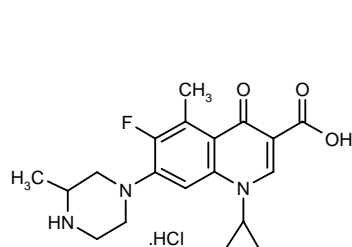
(6)



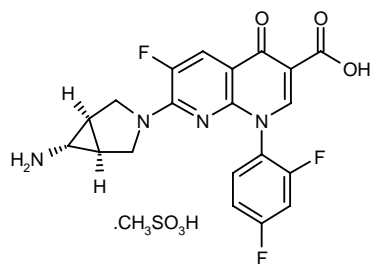
(7)



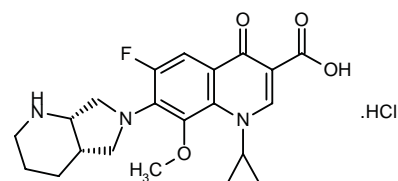
(8)



(9)



(10)



(11)

<sup>1</sup>Naphthyridine. <sup>2</sup>Cream and lotion for treatment of acne. <sup>3</sup>Withdrawn October 1999. \*Due to hepatic toxicity, use is restricted to otherwise untreatable infections.

Table II: Chemical structures of fluoroquinolone antibacterial drugs under development (Prous Science Ensemble database).

**NDA filed**

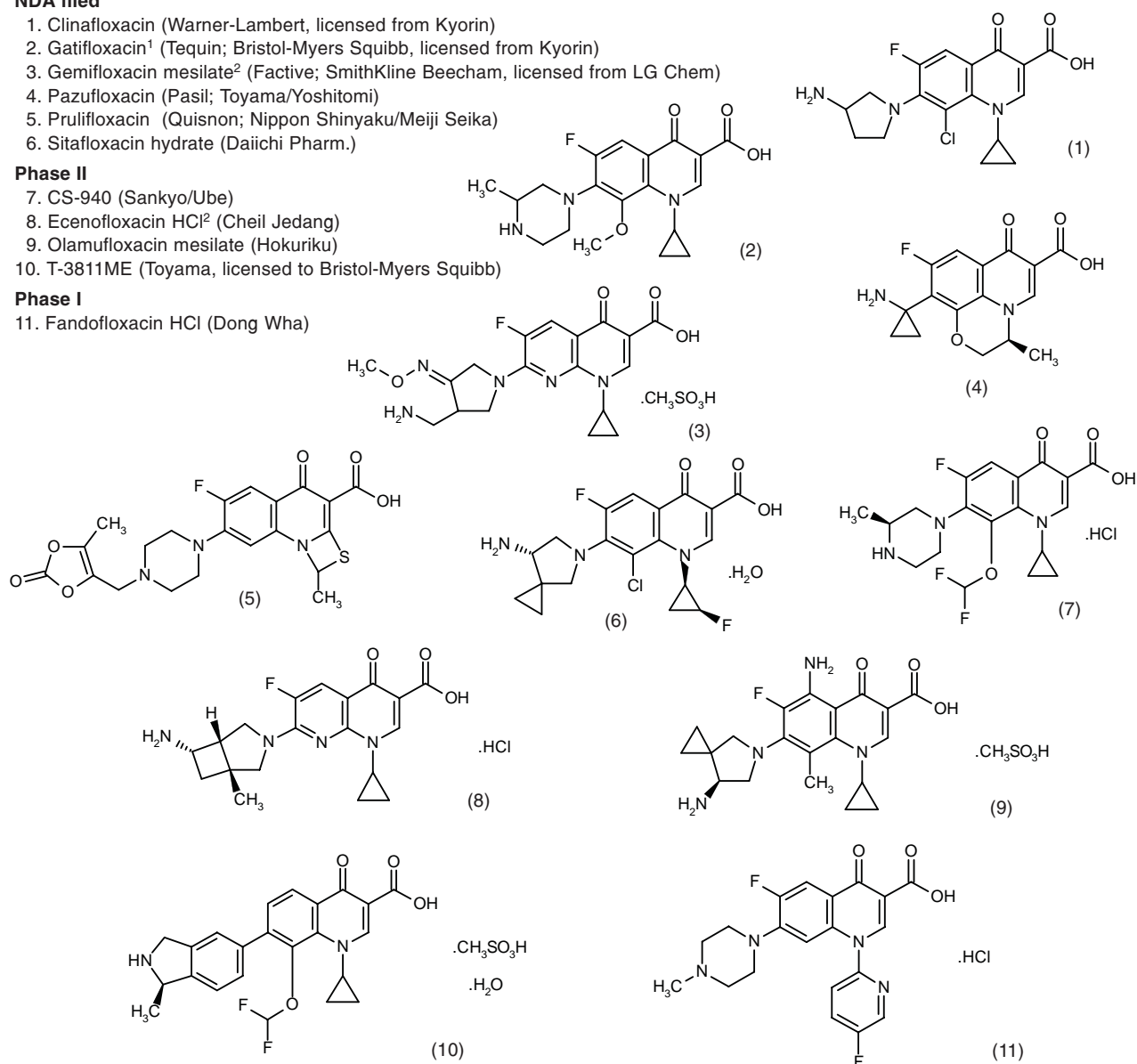
1. Clinafloxacin (Warner-Lambert, licensed from Kyorin)
2. Gatifloxacin<sup>1</sup> (Tequin; Bristol-Myers Squibb, licensed from Kyorin)
3. Gemifloxacin mesilate<sup>2</sup> (Factive; SmithKline Beecham, licensed from LG Chem)
4. Pazufloxacin (Pasil; Toyama/Yoshitomi)
5. Prulifloxacin (Quisnon; Nippon Shinyaku/Meiji Seika)
6. Sitafloxacin hydrate (Daiichi Pharm.)

**Phase II**

7. CS-940 (Sankyo/Ube)
8. Ecenofloxacin HCl<sup>2</sup> (Cheil Jedang)
9. Olamufloxacin mesilate (Hokuriku)
10. T-3811ME (Toyama, licensed to Bristol-Myers Squibb)

**Phase I**

11. Fandofloxacin HCl (Dong Wha)



<sup>1</sup>Approved in the U.S. in December 1999. <sup>2</sup>Naphthyridine.

**Introduction**

Since the discovery of nalidixic acid in 1962, the quinolone antibacterials have emerged as a major class of anti-infective agents. In the 1980s the fluoroquinolone antibacterials were discovered and were shown to be considerably more potent and to have a broader spectrum of activity than nalidixic acid. Fluoroquinolones are bactericidal and act by inhibiting bacterial DNA gyrase, which is responsible for maintaining the structure of bacterial DNA. Several quinolones have been launched (Table I), while others are progressing through clinical trials (Table II).

Although less potent than the fluoroquinolones, nalidixic acid remains in clinical usage mainly against common Gram-negative pathogens encountered in the urinary and gastrointestinal tracts.

In the search for new antibacterial agents, a series of *N*<sup>1</sup>-cyclopropyl-4-quinolones bearing an isoindolin-5-yl substituent at the 7-position was synthesized by scientists at Toyama. The result of their research was the discovery that several des-F(6) compounds exhibited potent antibacterial activity against Gram-positive and Gram-negative bacteria, comparable to that of the F(6)-substituted counterparts, while having lower acute toxicity in

mice. From this series of compounds, T-3811 was selected for further evaluation (2).

### Pharmacological Actions

The *in vitro* activity of T-3811 (the anhydrous free base of T-3811ME) has been compared to that of reference fluoroquinolones (trovafloxacin, ciprofloxacin and levofloxacin) against a battery of 1160 clinical isolates of Gram-positive and Gram-negative bacteria. T-3811 demonstrated a wide range of antimicrobial activity, including activity against *Chlamydia trachomatis*, *Mycoplasma pneumoniae* and *Mycobacterium tuberculosis*. It had particularly potent activity against Gram-positive cocci, with MIC<sub>90</sub> values in the range of 0.025-6.25 µg/ml. T-3811 was more potent than the reference compounds against methicillin-resistant *Staphylococcus aureus* and streptococci, including penicillin-resistant strains of *Streptococcus pneumoniae*, as well as against *Haemophilus influenzae*, *Moraxella catarrhalis* and *Legionella* spp. (MIC<sub>90</sub>s = 0.0125-0.1 µg/ml). Its activity against *Clostridium trachomatis* and *M. pneumoniae* was also superior to that of the reference compounds. Title compound retained its potent activity against quinolone-resistant Gram-positive cocci with GyrA and ParC (GrIA) mutations. Its activity against *Enterobacteriaceae* and nonfermentative Gram-negative rods was similar to that of trovafloxacin, while it had more potent activity than trovafloxacin against *M. tuberculosis* (MIC<sub>90</sub> = 0.0625 µg/ml). T-3811 also demonstrated potent activity against anaerobic bacteria, including *Bacteroides fragilis* and *Clostridium difficile* (3).

*In vivo* in mice with experimental systemic infections produced by GrIA mutant and GrIA GyrA double-mutant strains of *S. aureus*, T-3811ME demonstrated excellent therapeutic efficacy following oral (ED<sub>50</sub> = 0.0189 mg/mouse) or subcutaneous (ED<sub>50</sub> = 0.622 mg/mouse) administration. The activity of T-3811ME in this model was 4.3-, 30.3- and 70.4-fold more potent than that of trovafloxacin, levofloxacin and ciprofloxacin, respectively. Similar efficacy was observed in mice with experimental PRSP-induced pneumonia treated with the compound by the oral or s.c. route (ED<sub>50</sub>s = 0.0278 and 0.0266 mg/mouse, respectively) (3).

In another *in vivo* study, the effect of T-3811ME was evaluated in hamsters with experimental pneumonia caused by *M. pneumoniae*. Beginning on the seventh day after infection, mice were administered once-daily T-3811 (20 mg/kg p.o.), trovafloxacin or clarithromycin for 5 days. Following administration of the study drug at this dose, C<sub>max</sub> of T-3811ME in the lung and BALF of infected hamsters remained at levels 67- and 2.7-fold higher than the MIC<sub>90</sub> for *M. pneumoniae* for up to 6 h postdosing. The study drug was more effective than either trovafloxacin or clarithromycin at 2 and 5 days. On day 7, untreated control mice had peribronchial cellular infiltration and cellular infiltration into the alveolar septa or alveolar space. T-3811-treated mice also showed signs of slight peri-

bronchial cellular infiltration but no cellular infiltration into the alveolar septa or space. Cellular infiltration into the alveolar septa and/or space were observed in trovafloxacin- and clarithromycin-treated hamsters (4).

The compound's efficacy has also been evaluated and compared to ciprofloxacin and ceftriaxone in a rabbit model of experimental meningitis caused by penicillin-resistant *S. pneumoniae*. At doses of 20 mg/kg for T-3811ME and ciprofloxacin and 100 mg/kg for ceftriaxone, given i.v. 18 h after intracisternal injection of bacteria, concentrations of T-3811ME and ceftriaxone, but not ciprofloxacin, in the cerebrospinal fluid (CSF) exceeded the MIC against penicillin-resistant *S. pneumoniae* for 6 h. Reductions in log CFU/ml in CSF at 2 and 6 h were significantly greater for T-3811ME than for the reference compounds (5).

The comparative antimicrobial profiles of T-3811 and representative fluoroquinolones are summarized graphically in Figure 1.

### Pharmacokinetics and Metabolism

The pharmacokinetics of T-3811 were studied in mice, rats and dogs following the administration of a single oral or i.v. dose of 5 mg/kg, and were compared to those of ciprofloxacin and trovafloxacin. The oral bioavailability of the study drug was excellent (96%, 73% and 81% in mice, rats and dogs, respectively), and was consistently superior to that of ciprofloxacin (49%, 43% and 55%, respectively). The AUC for T-3811 was also greater than that of ciprofloxacin following both oral and i.v. dosing. Drug concentrations in lung, kidney and liver tissues following either oral or i.v. dosing were greatest with T-3811. Urinary recovery of T-3811 after oral administration was 14-15%, compared to < 2.3% for trovafloxacin and 25-29% for ciprofloxacin. In addition to unchanged drug, two major metabolites of T-3811 were recovered in the urine and bile: the *N*-sulfonic acid (37%) and the glucuronide (17%). *In vitro* serum protein binding values for T-3811 in mouse, rat, dog and human serum were much greater than those of ciprofloxacin and similar to those of trovafloxacin (6).

### Toxicity

Convulsions induced by inhibition of GABA receptor binding represent one of the severe side effects of quinolones. Other frequently encountered side effects include nausea and vomiting and the loss of coordination and balance. Thus, the CNS toxicity of T-3811ME has been evaluated and compared to that of other quinolones in several assays. As assessed in biphenylacetic acid-treated mice and fenbufen-pretreated mice, the new quinolone was less proconvulsant than other quinolones. It was also associated with less emetic activity in dogs and with fewer effects on motor coordination in mice than trovafloxacin. T-3811ME was the least active compound tested as regards inhibition of GABA receptor binding (7).

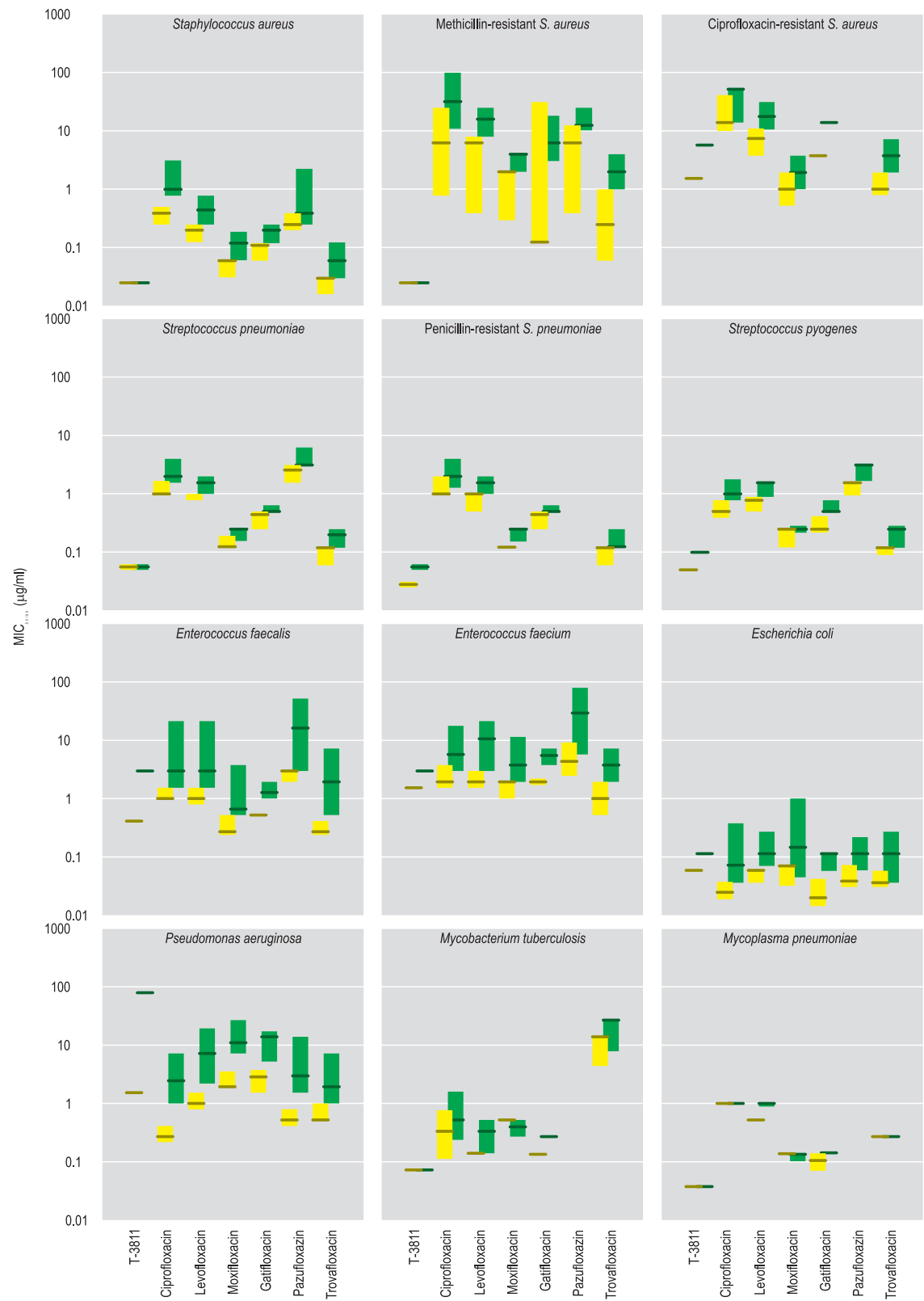


Fig. 1. Comparative *in vitro* antimicrobial activity of T-3811 and reference fluoroquinolones against representative Gram-positive and Gram-negative bacteria. Data from individual studies have been plotted using a box-type graph, which displays median (horizontal lines) and 25-75 percentiles (color bars) for MIC<sub>50</sub> (yellow) and MIC<sub>90</sub> (green) values. Data have been extracted from 244 references included in the Prous Science MFlne database.

Many fluoroquinolone antibacterials are known to cause cavitation and erosion of articular cartilage when administered systemically to young animals and, as such, their use in children is usually limited. Thus, it was considered appropriate to study the articular toxicity of TE-3811ME *in vivo* in juvenile dogs administered multiple i.v. doses (30 mg/kg/day x 7 days), as well as to assess its direct toxicity *in vitro* in isolated growing canine chondrocytes. No cartilage changes were observed *in vivo* in any of the 3 dogs administered T-3811ME, in contrast to trovafloxacin, which induced knee joint lesions in 1/3 treated dogs. T-3811ME did inhibit chondrocyte proliferation during the growing phase to some extent, although this effect was less pronounced than that of trovafloxacin. In the plateau phase (after 6 days in culture), trovafloxacin inhibited mitochondrial dehydrogenase activity and prostaglandin secretion, while T-3811ME had only a slight inhibitory effect. Overall, the articular toxicity of T-3811ME was weaker than that of trovafloxacin, even at higher intracellular concentrations (8).

The potential of T-3811 to cause other adverse reactions sometimes associated with fluoroquinolone administration – e.g., hypotension, histamine release, inhibition of the CYP1A2 activity in human generated microsomes and local injection-site skin reactions – has also been studied and compared to that of other marketed and investigational fluoroquinolones. In every instance, the title compound was less likely to induce adverse events than any of the reference quinolones (9).

The oral LD<sub>50</sub> value for T-3811 was determined to be greater than 5 g/kg in male rats, while the i.v. LD<sub>50</sub> value in male rats and mice was 250-300 mg/kg (10).

## Clinical Studies

Bristol-Myers Squibb, which is developing T-3811ME under license from Toyama, has initiated phase II clinical testing of the drug (which it has designated BMS-284756). Toyama, which retains rights in Japan, is conducting phase I studies in that country (11).

## Manufacturer

Toyama Chemical Co., Ltd. (JP); licensed to Bristol-Myers Squibb (US).

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