mL of cold buffer. The radioactivity associated with the filter was determined by liquid-scintillation spectrometry. Nonspecific binding was defined with 60 μM MK-801. IC_{50} were determined using a logit-log transformation of the binding data.

[^{3}H]Glycine Binding Assay. [^{3}H]Glycine binding to the NMDA receptor associated strychnine-insensitive recognition site was performed as previously described.[30]

Registry No. **7**, 1067-87-4; (*Z*)-**8**, 129920-47-4; (*E*)-**8**, 129920-48-5; (*Z*)-**9**, 129920-49-6; (*E*)-**9**, 129920-50-9; **10a**, 129920-51-0; **10b**, 129920-52-1; **11a**, 129920-53-2; **11b**, 129920-54-3; **12a** (diastereomer 1), 129920-55-4; **12a** (diastereomer 2), 130008-24-1; **12b** (diastereomer 1), 130008-31-0; **12b** (diastereomer 2), 130008-32-1; **13a** (diastereomer 1), 129920-81-6; **13a** (diastereomer 2), 130008-33-2; **13b** (diastereomer 1), 130008-34-3; **13b** (diastereomer 2), 130008-35-4; **14**, 15916-48-0; **15**, 129920-56-5; **16**, 129920-57-6; **17**, 129920-58-7; **18**, 129920-59-8; **19**, 129920-84-9; **20**, 129920-60-1; **21**, 129920-61-2; **22**, 129920-62-3; **23**, 129920-85-0; **24**, 127515-28-0; (*Z*)-**25** (diastereomer 1), 129920-87-2; (*Z*)-**25** (diastereomer 2), 130008-45-6; (*E*)-**25** (diastereomer 1), 130008-46-7; (*E*)-**25**, 130008-47-8; (*Z*)-**26** (diastereomer 1), 129920-86-1; (*Z*)-**26** (diastereomer 2), 130008-42-3; (*E*)-**26** (diastereomer 1), 130008-43-4; (*E*)-**26** (diastereomer 2), 130008-44-5; **27a**, 5162-44-7; **27c**, 1119-51-3; **28a**, 129920-63-4; **28b**, 129920-64-5; **28c**, 129920-65-6; **28d**, 129920-66-7; **29a**, 129920-67-8; **29b**, 129920-68-9; **29c**, 129920-69-0; **29d**, 129920-70-3; **30a**, 129920-71-4; **30b**, 129920-72-5; **30c**, 129920-73-6; **30d**, 129920-74-7; **31a**, 129920-75-8; **31b**, 129920-76-9; **31c**, 129920-77-0; **31d**, 129920-78-1; **32a** (diastereomer 1), 129920-79-2; **32a** (diastereomer 2), 130008-25-2; **32b** (diastereomer 1), 130008-26-3; **32b** (diastereomer 2), 130008-27-4; **32c** (diastereomer 1), 129920-80-5; **32c** (diastereomer 2), 130008-28-5; **32d** (diastereomer 1), 130008-29-6; **32d** (diastereomer 2), 130008-30-9; **33a** (diastereomer 1), 129920-82-7; **33a** (diastereomer 2), 130008-36-5; **33b** (diastereomer 1), 130008-37-6; **33b** (diastereomer 2), 130008-38-7; **33c** (diastereomer 1), 129920-83-8; **33c** (diastereomer 2), 130008-39-8; **33d** (diastereomer 1), 130008-40-1; **33d** (diastereomer 2), 130008-41-2; NMDA, 6384-92-5; $BrCH_2C{-}H{=}CH_2$, 106-95-6; $N_2{=}CHCOOEt$, 623-73-4; $N_2{=}C(COOMe)_2$, 6773-29-1; $N_2{=}CHPO_3Me_2$, 27491-70-9.

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Synthesis, Antibacterial Activities, and Pharmacological Properties of Enantiomers of Temafloxacin Hydrochloride[1]

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Temafloxacin hydrochloride [(±)-7-(3-methylpiperazin-1-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride] is a potent member of the 4-pyridone-3-carboxylic acid class of antibacterial agents and is currently under clinical development as a broad-spectrum antimicrobial agent. It is a racemate having a chiral center at the C_3 of the 7-piperazin-1-yl group. The two enantiomers were synthesized and tested for their antibacterial activities. Although no difference in in vitro antibacterial activities was observed, a minor difference in in vivo antibacterial activities was observed. However, they both exhibited similar pharmacological profiles.

In recent years, many clinically important antibacterial agents (such as ciprofloxacin (**1**)[2] and norfloxacin (**2**)[3]) having the 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic acid moiety and collectively known as quinolones have been discovered.[4] These agents have been shown to inhibit the topoisomerase enzyme DNA gyrase.[5,6] Hence, it may be expected that chirality in the quinolone molecule can have a great impact on the biological activity. Nearly all clinically useful quinolones developed to date, however, are either achiral or racemic mixtures. The *S* enantiomers of ofloxacin (**3**)[7,8] and S-25930 (**4**)[9] have recently been reported to possess greater biological activities (10–100-fold) than their antipodes. The *R* enantiomer of 7-(2-substituted-pyrrolidin-1-yl)quinolone derivative **5a** possesses 10–60-fold greater potency than the *S* enantiomer.[10] Minor differences in activity are observed with the two enantiomers of the 7-(3-aminopyrrolin-1-yl)-naphthyridine derivative **5b**.[11] The enantiomers of the 3-[(ethylamino)methyl]pyrrolidin-1-yl derivative **6**, however, were reported to have similar biological activity.[12]

Temafloxacin hydrochloride (**7**) [(±)-7-(3-methylpiperazin-1-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride] is a potent quinolone antibacterial agent. It is currently under clinical development and a NDA has been filed in the U.S. It possesses excellent activity against both Gram-positive and Gram-negative bacteria.[12] Temafloxacin is a racemate having a chiral center at C-3 of the 7-piperazin-1-yl group. Because of the excellent biological activity of **7** and the presence of a chiral center, both enantiomers were synthesized to evaluate their potential differences in biological, pharmacological, and toxicological properties. In this paper, we now report the synthesis and properties of the enantiomers of temafloxacin hydrochloride.

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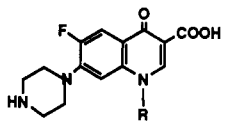
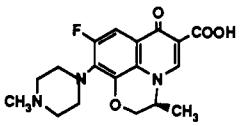


1 R = c-C_3H_5
2 R = C_2H_5



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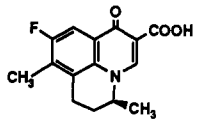

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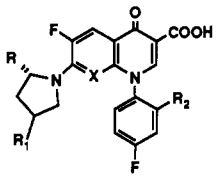
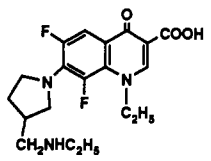
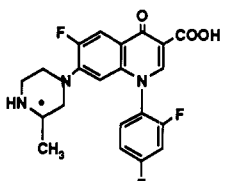


5a X=CH, R=CH_2OH, R_1=R_2=H

5b X=N, R=H, R_1=NH_2, R_2=F



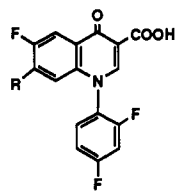
6



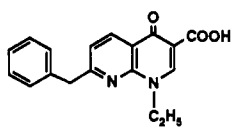
7

Chemistry

The general method used for the preparation of *N*-arylfluoroquinolones involving an intramolecular nucleophilic displacement cyclization reaction has been previously described.[14] However, the displacement of the known key intermediate 6-fluoro-7-chloro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**8**) by a substituted amine provided the 7-aminoquinolone derivative in low yield. In order to improve the synthetic yield, we chose a modified synthetic route utilizing 6,7-difluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**9**) as the key intermediate.[15] The C-7 fluorine atom in **9** was found to be a much better leaving group than the C-7 chlorine atom in **8**. Hence, the side reaction of the displacement of the fluorine atom in the 2,4-difluorophenyl group as well as the C-6 fluorine group by the amine in **9** was avoided.



8 R = Cl
9 R = F



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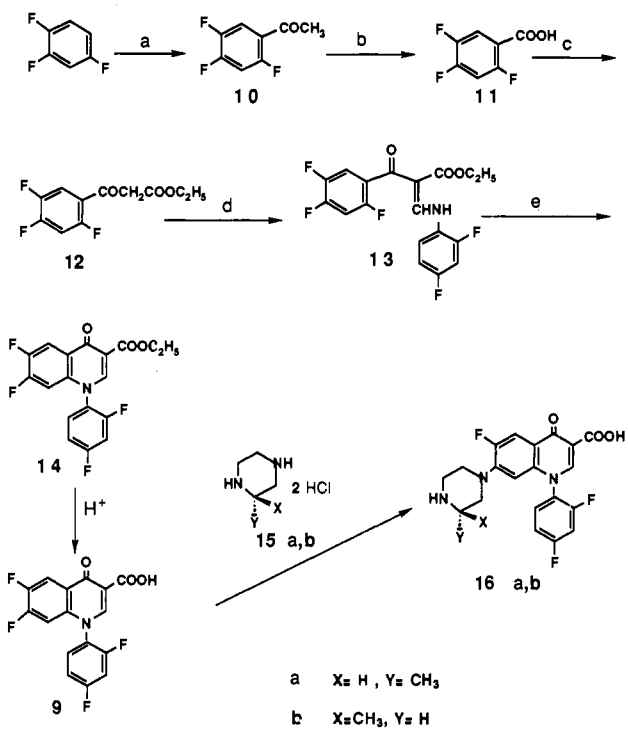
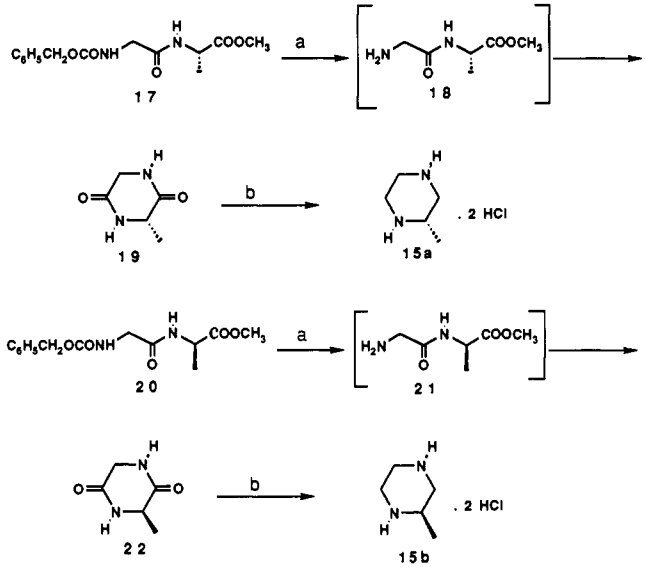
Friedel–Crafts acylation of 1,3,4-trifluorobenzene with acetyl chloride in the presence of aluminum chloride yielded 2,4,5-trifluoroacetophenone (**10**) (bp 69 °C/8 mmHg; 75%). Oxidation of **10** with 5.25% sodium hypochlorite and sodium hydroxide solution gave the 2,4,5-trifluorobenzoic acid (**11**) (mp 96–98 °C; 94%). The acid **11** was converted into the acid chloride upon treatment with thionyl chloride. It was then displaced by the lithium monoethyl malonate dianion to give the β-keto ester **12**. Reaction of this **12** with triethyl orthoformate in acetic anhydride yielded an enol ether intermediate, which upon evaporation of solvent to dryness was allowed to react with a slight excess of an 2,4-difluoroaniline in methylene chloride at room temperature to give the enamino keto ester **13** (mp 85–87 °C; 61%). Cyclization of **13** with sodium hydride yielded ethyl 1-(2,4-difluorophenyl)-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**14**) (mp

Scheme I



a) $CH_3COCl/AlCl_3$ b) NaOCl/NaOH; HCl c) $SOCl_2$; $CH_2(COOC_2H_5)COOH$/n-BuLi; HCl d) $CH(OC_2H_5)_3/Ac_2O$; o,p-difluoroaniline e) NaH/THF

Scheme II



a) H_2; Pd/C b) BH_3.THF

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(15) The synthesis of racemate temafloxacin utilizing compound **9** has been presented at the 26th ICAAC, Sept 28, New Orleans, 1986 (Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Maleczka, R. E.; Klock, P.; Shen, L.; Patel, J.; Pernet, A.). An independent synthesis of temafloxacin utilizing similar intermediates was also reported (Narita, H.; Konishi, Y.; Nitta, J.; Nagaski, H.; Kobayashi, Y.; Watanabe, Y. *J. Pharm. Sci. Jpn.* **1986**, *106*, 795).

Table I. In Vitro Antibacterial Activities of Temafloxacin (Tema) and Its Enantiomers

organism	MIC, μg/mL S(-)-Tema	R(+)-Tema	Tema
Staphylococcus aureus ATCC 6538P	0.1	0.1	0.1
Staphylococcus aureus A5177	0.2	0.2	0.2
Staphylococcus aureus 45	0.2	0.2	0.2
Staphylococcus aureus 45 RAR2	0.39	0.39	0.39
Staphylococcus aureus 642A	0.2	0.2	
Staphylococcus aureus NCTC 10649	0.1	0.1	
Staphylococcus aureus CMX 553	0.2	0.2	0.2
Staphylococcus epidermidis 3519	0.2	0.2	0.2
Micrococcus luteus ATCC 9341	3.1	3.1	3.1
Micrococcus luteus ATCC 4698	1.56	1.56	1.56
Enterococcus faecium ATCC 8043	0.78	0.78	0.78
Streptococcus bovis A5169	1.56	1.56	1.56
Streptococcus agalactiae CMX 508	0.78	0.78	0.78
Streptococcus pyogenes EES61	0.39	0.39	0.39
Streptococcus pyogenes 930 Const	0.39	0.39	0.78
Streptococcus pyogenes 2548 INDUC	0.2	0.2	0.39
Escherichia coli Juhl	0.05	0.05	0.05
Escherichia coli SS	0.002	0.01	0.005
Escherichia coli DC-2	0.78	0.78	0.39
Escherichia coli H560	0.02	0.05	0.05
Escherichia coli KNK437	0.39	0.39	0.39
Enterobacter aerogenes ATCC13048	0.1	0.1	0.1
Klebsiella pneumoniae ATCC 8045	0.1	0.1	0.05
Providencia stuartii CMX 640	6.2	3.1	3.1
Pseudomonas aeruginosa BMH10	0.2	0.39	0.39
Pseudomonas aeruginosa A5007	0.39	0.78	0.39
Pseudomonas aeruginosa K799/WT	0.39	0.39	0.39
Pseudomonas aeruginosa K799/61	0.05	0.05	0.05
Pseudomonas cepacia 2961	6.2	12.5	12.5
Acinetobacter sp. CMX 669	0.1	0.1	0.1

204–205 °C; 77%). Hydrolysis of **14** yielded the key intermediate 6,7-difluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**9**). Displacement of **9** with the respective optically pure 2-methylpiperazine dihydrochlorides (**15a** or **15b**) in pyridine yielded the desired (*S*)-(−)-temafloxacin hydrochloride (**16a**) (mp >300 °C; 49%) and (*R*)-(±)-temafloxacin hydrochloride (**16b**) (mp >300 °C; 42%) (Scheme I).

The optically pure (*S*)-2-methylpiperazine was prepared earlier[14] in an extremely low yield by sublimation of glycyl-L-alanine followed by reduction of the diketopiperazine with sodium dimethoxyethoxydihydroaluminate. Because of the very low yield associated with this reduction step, an optical resolution was used as a practical synthesis of 2-methylpiperazine enantiomers, but only partial resolution was achieved.[16] The requisite optically pure enantiomers were prepared in good yield, however, by a modified route as outlined in Scheme II. Deprotection of carbobenzoxyglycyl-L-alanine methyl ester (**17**) by hydrogenolysis yielded glycyl-L-alanine methyl ester (**18**) intermediate, which, without isolation, was allowed to cyclize to give the diketopiperazine **19** (mp 246–247 °C; 83%). Reduction of **19** (*S* enantiomer) with borane–tetrahydrofuran complex yielded the (*S*)-(−)-2-methylpiperazine dihydrochloride (**15a**) (mp >300 °C; 53%). By use of a similar synthetic sequence, the (*R*)-(+)-2-methylpiperazine dihydrochloride (**15b**) was prepared from carbobenzoxyglycyl-D-alanine methyl ester. By use of the above synthetic route, racemization is normally not expected to occur. The specific rotations for these two enantiomers ($[\alpha]^{25.5}_D$ = −2.73 (H_2O) for **15a** and $[\alpha]^{26}_D$ = +2.63 (H_2O) for **15b**), however, showed a slight difference. Although this difference may be the result of experimental error, a slight partial racemization during their syntheses may not be totally excluded.

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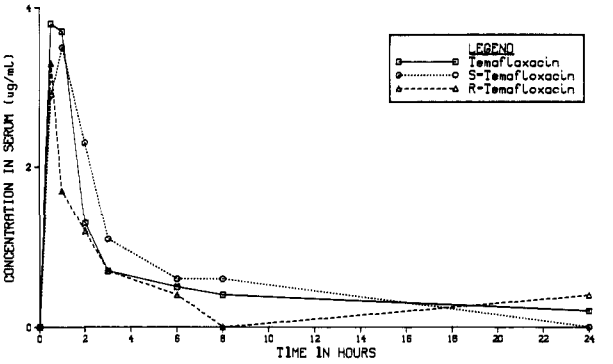

Figure 1. Serum concentrations of temafloxacin and its enantiomers following a single oral administration of 25 mg/kg in mice.

Biological Results and Discussion

Microbiology. The comparative in vitro antibacterial activity of the two enantiomers of temafloxacin (Tema) against representatives of Gram-positive and Gram-negative bacteria is shown in Table I. Data for racemic tefloxacin is provided for comparison. The in vitro antibacterial activities are reported as minimum inhibitory concentration (MIC) in μg/mL. The MIC's were determined by the 2-fold agar dilution in brain–heart infusion agar. It can be seen from Table I that temafloxacin and its *R* and *S* enantiomers possess essentially identical antibacterial potency in vitro. The magnitude of the differences (3 out of 30 organisms) is within the margin of experimental error.

In order to determine the inherent activity, the enantiomers were assayed[17] for supercoiling inhibition activity with DNA gyrase isolated from *Escherichia coli* H560. The IC_{50} values in μg/mL for the **16a** (*S* enantiomer) and **16b** (*R* enantiomer) are 0.52 and 0.76, respectively. Within the experimental error, the two enantiomers possess similar activity at the enzymatic level.

The in vivo potency of the *R* and *S* enantiomers as well as the racemate (temafloxacin) determined by the mouse protection test is shown in Table II. The potency is given in ED_{50} values which are expressed as the total dose of compound in mg/kg required to protect 50% the mice challenged intraperitoneally with organism indicated. The mice were treated subcutaneously (sc) or orally (po) with a specific amount of the test compound divided equally to be administered at 1 and 5 h after infection. When the 95% confidence limits are taken into consideration, the data showed that in vivo, following both oral or subcutaneous administration to the mice, both (*S*)-(−)-temafloxacin (**16a**) and (*R*)-(+)-temafloxacin (**16b**) possess comparable potency against *Staphylococcus aureus* NCTC 10649, *Pseudomonas aeruginosa* A5007, and *Escherichia coli* Juhl. Against *Streptococcus pneumoniae* 6303, the *S*-(−)-enantiomer is slightly more active than the *R*-(+)-enantiomer. (±)-Temafloxacin possesses intermediate potency. It is possible that the slightly higher in vivo potency of (*S*)-(−)-temafloxacin is the result of better pharmacokinetics and metabolism in the animal model, since all the test compounds are equally potent in vitro.

The plasma concentrations of (*R*)-(+)- and (*S*)-(−)-temafloxacin after an oral dose of 25 mg/kg of the test compounds administered to mice is given in Figure 1. The *S*-(−) enantiomer provided an area under the serum curve (AUC) of 9.4 mcg/hr/mL while the *R*-(+) enantiomer gave an AUC of 4.7 μg h^{-1} mL^{-1}. The plasma concentration of

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Table II. Mouse Protection Test of Temafloxacin (Tema) and Its Enantiomers

test organism dose	compound[a]	ED_{50}, mg/kg (95% confidence limits) route	test 1	test 2
S. aureus NCTC 10649 (100×LD_{50})	*S*(−)-Tema	sc	0.9 (0.6–1.4)	1.5 (1.0–2.2)
	R(+)-Tema	sc	2.0 (1.3–3.0)	2.8 (1.8–4.2)
	Tema	sc	1.5 (1.0–2.2)	1.3 (0.8–2.2)
	S(−)-Tema	po	3.1 (1.8–5.3)	3.6 (2.1–6.3)
	R(+)-Tema	po	4.4 (2.5–7.7)	6.8 (4.3–10.7)
	Tema	po	6.0 (3.8–9.5)	3.2 (1.6–6.3)
S. pneumoniae 6303 (100×LD_{50})	*S*(−)-Tema	sc	4.7 (3.0–7.6)	
	R(+)-Tema	sc	17.2 (11.3–26.3)	
	Tema	sc	6.2 (3.9–9.9)	
	S(−)-Tema	po	5.9 (3.8–9.1)	
	R(+)-Tema	po	19.5 (11.6–33.0)	
	Tema	po	10.2 (6.4–16.4)	
E. coli Juhl (100×LD_{50})	*S*(−)-Tema	sc	0.3 (0.2–0.5)	0.5 (0.3–0.8)
	R(+)-Tema	sc	1.2 (0.7–2.1)	0.9 (0.5–1.8)
	Tema	sc	0.5 (0.3–0.8)	0.6 (0.4–1.0)
	S(−)-Tema	po	2.3 (1.7–3.1)	2.5 (1.9–3.2)
	R(+)-Tema	po	3.6 (1.8–7.5)	4.5 (2.3–8.9)
	Tema	po	3.7 (2.1–6.5)	5.0 (3.2–7.9)
P. aeruginosa A5007 (100×LD_{50})	*S*(−)-Tema	sc	10.3 (6.6–16.3)	10.5 (5.7–19.2)
	R(+)-Tema	sc	10.7 (6.6–17.2)	16.5 (10.5–25.8)[b]
	Tema	sc	10.3 (6.6–16.3)	12.5 (7.9–19.8)
	S(−)-Tema	po	19.9 (12.8–31.1)	25.0 (15.8–39.6)
	R(+)-Tema	po	62.0 (31.3–122.9)	51.3 (30.7–85.6)[b]
	Tema	po	26.6 (13.7–51.4)	26.6 (13.7–51.4)

[a] Each compound was tested at three dose with 10 animals per dose. ED_{50} is the median dose calculated from cumulative mortalities. [b] Infecting dose 1000×LD_{50}.

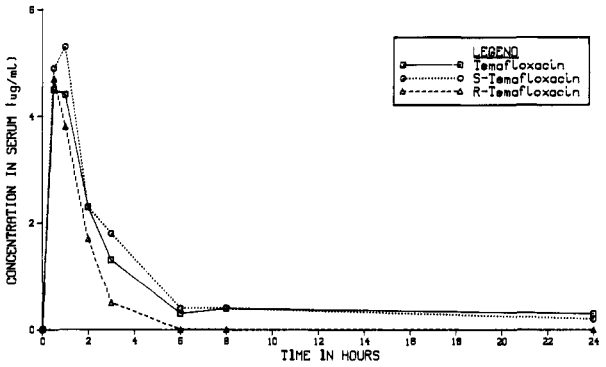

Figure 2. Serum concentrations of temafloxacin and its enantiomers following a single subcutaneous administration of 25 mg/kg in mice.

R and *S* enantiomers at a single subcutaneous dose of 25 mg/kg to mice is shown in Figure 2. The AUCs for *S* and *R* enantiomers were 11.2 and 5.7 $\mu g\ h^{-1}\ mL^{-1}$, respectively. As indicated in Figures 1 and 2, the *S*-(−) enantiomer had almost 2-fold greater area under the serum curve than the *R*-(+) enantiomer. Although the peak serum level for the two enantiomers are almost identical, the *S*-(−) enantiomer persisted at a higher concentration up to 8 h after dosing. The differences may account for the slight difference in in vivo efficacy in the mouse protection tests.

Solubility Studies. The slight difference in pharmacokinetic properties with the two enantiomers may be due to their differences in physical properties. The solubilities and the partition coefficients in octanol/water (log *P*) of (±)-temafloxacin and its enantiomers were determined as shown in Table III. However, both enantiomers exhibit essentially identical solubility and log *P* values. It should be noted that racemic temafloxacin has twice the solubility value as its enantiomers at physiological pH which may be of practical importance.

Pharmacology. Central nervous system (CNS) stimulation is a characteristic side effect of some members of quinolone antibacterials currently in clinical use. Mild headache, dizziness, sleep disturbance, or mood alterations are the common side effects. Seizures have been reported

Table III. Comparative Physical Properties of Temafloxacin and Its Enantiomers

compound	water solubility in water, mg/mL	water solubility[a] at pH 7.5, mg/mL	log *P*[b]
S enantiomer	5.5	0.37	−0.27
R enantiomer	5.9	0.36	−0.27
temafloxacin	6.5	0.67	−0.29

[a] Water solubility of test compound in 0.05 M pH 7.5 phosphate buffer. [b] The partition coefficients in octanol/water at pH 7.5 buffer.

in small numbers of patients receiving ciprofloxacin[18] and enoxacin.[19] Amfonelic acid (**23**), a quinolone derivative, produced a marked CNS stimulant effect on locomotor activity.[20] With the availability of both temafloxacin enantiomers, the potential CNS effects of (*R*)- and (*S*)-temafloxacins were evaluated in male Sprague-Dawley rats by a measurement of locomotor activity. The effects of (*R*)- and (*S*)-temafloxacin and oxolinic acid on the spontaneous motor activity of rats are shown in Table IV. The enantiomers were administered at doses of 30, 100, and 300 mg/kg, intraperitoneally (ip), and oxolinic acid was administered at doses of 30, 100, and 300 mg/kg, orally (po). At 30 min after the administration of the test compounds or a volume dose of the vehicle (methylcellulose), the rats were placed into individual circular activity chambers. Spontaneous locomotor activity measurements were derived from the interruption of photocell beams which are strategically placed within the chambers. Activity counts were recorded at 15 and 60 min after the start of the test. The treatments were administered on a random basis. At intraperitoneal doses at 30 and 100 mg/kg, no significant effects were seen with either enantiomer of temafloxacin. However, at an extremely high ip dose of 300 mg/kg, both enantiomers significantly reduced locomotor activity at

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Table IV. Effects of Intraperitoneal Administration of (*R*)- and (*S*)-Temafloxacin on the Spontaneous Locomotor Activity of Rats

treatment	dose, mg/kg	route	mean activity counts ± SEM 0–15 min	0–60 min
vehicle		ip	736 ± 46	962 ± 59
(*R*)-temafloxacin	30	ip	699 ± 57	863 ± 92
	100	ip	673 ± 90	822 ± 92
	300	ip	183 ± 70[a]	291 ± 75[a]
vehicle		ip	571 ± 70	922 ± 115
(*S*)-temafloxacin	30	ip	549 ± 52	751 ± 99
	100	ip	386 ± 64	763 ± 97
	300	ip	270 ± 47[a]	354 ± 51[a]
methamphetamine	1	ip	1462 ± 118[a]	3728 ± 478[a]
chlorpromazine	15	ip	31 ± 28[a]	68 ± 37[a]
vehicle		po	819 ± 55	1087 ± 106
oxolinic acid	30	po	972 ± 65	1746 ± 165[a]
	100	po	1074 ± 79	2561 ± 4541[a]
	300	po	1031 ± 87	3085 ± 391[a]

[a] Denotes that the group ($N = 6$ with the exception of oxolinic acid where $N = 12$) is significantly different from vehicle at 0.05 level of confidence using one-way analysis of variance and the Newman–Keuls procedure[21] on transformed data.

both the 15- and 60-min measurements. In general, both compounds had similar effects on the spontaneous locomotor activity of rats. In contrast, oxolinic acid at 30, 100, and 300 mg/kg, po produced significant increase in locomotor activity as shown in Table IV. The effects of methamphetamine (1 mg/kg, ip), a known CNS stimulant, and chlorpromazine (15 mg/kg, ip), a known CNS depressant on locomotor activity was also included for comparison. Neither enantiomers of temafloxacin showed the marked stimulant or depressant activity associated with these centrally active drugs.

Summary of Results. The enantiomers of temafloxacin (7) have been synthesized. They were evaluated in vitro against both Gram-positive and Gram-negative bacteria. Their in vivo efficacy, pharmacokinetic properties, and CNS pharmacology were assessed. (±)-Temafloxacin was shown to be as active as its *R*-(+) or *S*-(−) enantiomers in vitro. A slightly better pharmacokinetic property was observed for (*S*)-(−)-temafloxacin in mice. It remains to be seen whether other animal species will provide similar differences. Because the enantiomers have identical antibacterial potency in vitro, as well as similar CNS activity on spontaneous locomotor activity in rats, and because the racemate has higher water solubility, the continuation of clinical development for the racemic temafloxacin remains appropriate.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were taken in a Thomas-Hoover capillary apparatus and was uncorrected. NMR spectra were determined on a General Electric GN-300 spectrometer operating at 300.1 MHz. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant ^{1}H NMR data are tabulated in the order: number of protons, multiplicity (s, singlet; d, doublet, t, triplet, q, quartet; m, multiplet; b, broad), coupling constant(s) and designation. The IR spectra were recorded on a Perkin-Elmer Model 710A infrared spectrometer. Mass spectra were obtained with a Hewlett-Packard 5985A mass spectrometer or a Kratos MS-50 instrument with EI source (70 eV). The IR, NMR and mass spectra data of all compounds were consistent with the assigned structures. Elemental analyses were obtained for all new compounds reported. Carbon, hydrogen, and nitrogen analyses (unless otherwise specified) were within ±0.4% of the theoretical values. Solutions were dried over magnesium sulfate. E. Merck silica gel (230–400 mesh) obtained from VWR Scientific was used for column chromatography, and yields of the reactions were not optimized. Elemental analyses were performed by the Abbott analytical department and IR, NMR, and mass spectra were recorded by the Abbott structural chemistry department.

2,4,5-Trifluoroacetophenone (10). Acetyl chloride (10.67 mL, 0.15 mol) was added dropwise to aluminum chloride (29.33 g, 0.22 mol) over a 15-min period. The reaction was exothermic with evolution of gas observed. The mixture was heated at 50 °C for 1 h. 1,2,4-Trifluorobenzene (13.2 g, 0.1 mol) was then added to the mixture over a 5-min period. The mixture was heated at 80 °C for 22 h. The mixture was cooled to room temperature and was poured into a mixture of 200 g of ice and 25 mL of concentrated hydrochloric acid mixture. After 0.5 h, it was extracted several times with methylene chloride. The combined methylene chloride extract was washed several times with dilute hydrochloric acid and then water. The solution was dried, filtered, and distilled at 69 °C (8 mmHg) to give 13 g of a mobile liquid (75% yield) of **10**. Anal. ($C_8H_5F_3$) C, H. NMR ($CDCl_3$): δ 2.67 (3 H, d, J_{H-F} = 6 Hz, CH_3), 7.06 (1 H, m, aromatic H), 7.78 (1 H, m, aromatic H).

2,4,5-Trifluorobenzoic Acid (11). Sodium hydroxide (42 g, 1.05 mol) was dissolved in 100 mL of water and was added to 2500 mL of commercial bleach (sodium hypochlorite, 5.25%) with rapid stirring. 2,4,5-Trifluoroacetophenone (91.5 g, 0.526 mol) was then added over a 25-min period. The reaction temperature was maintained below 25 °C. After 22 h, excess sodium bisulfite solution was added to decompose the excess of sodium hypochlorite until KI test paper was negative. The mixture was adjusted to pH 2 by the addition of concentrated hydrochloric acid. The precipitate was filtered and washed with water (2 × 200 mL) and dried. The aqueous phase was extracted several times with methylene chloride, dried, and evaporated to dryness to give a solid. The combined solid gave 86.73 g (94%) of 2,4,5-trifluorobenzoic acid (11), mp 96–98 °C. Anal. ($C_7H_3F_3O_2$) C, H. NMR (DMSO-d_6): δ 7.71 (1 H, m, aromatic H), 7.91 (1 H, m, aromatic H), 13.67 (1 H, bs, COOH).

Ethyl (2,4,5-Trifluorobenzoyl)acetate (12). After a mixture of 2,4,5-trifluorobenzoic acid (9.65 g, 54.8 mmol) and thionyl chloride (45 mL) was heated at 80 °C for 4 h, the solvent was removed by evaporation under reduced pressure, yielding a mobile oil (2,4,5-trifluorobenzoyl chloride). Monoethyl malonate (17 g, 128.8 mmol) and 5 mg of biquinoline were dissolved in 350 mL of dry tetrahydrofuran (THF), and the solution was cooled to −30 °C. A solution of approximately 2.6 M *n*-butyllithium in hexane was added until a pink color remained at −5 °C (99 mL). The suspension was then cooled to −50 °C. The acid chloride, obtained as described above, was dissolved in 70 mL of THF. This was added to the suspension dropwise. After 0.5 h, the dry ice bath was removed and the reaction mixture was allowed to warm up to room temperature. The reaction mixture was acidified with 250 mL of 1 N hydrochloric acid and was extracted with ether. The ether solution was dried and filtered. Evaporation of the solvent to dryness yielded an oil. This was purified by use of Kugelrohr distillation to give 13.2 g (98%) of **12**. NMR ($CDCl_3$): δ (two sets of signals), 1.22 (1.8 H, t, J = 7 Hz, ethyl CH_3), 1.27 (1.2 H, t, J = 7 Hz, ethyl CH_3), 3.30 (0.8 H, s, CH_2), 4.14 (1.2 H, q, J = 7 Hz, ethyl CH_2), 4.19 (0.8 H, q, J = 7 Hz, ethyl CH_2), 5.76 (0.6 H, s, vinyl H), 6.91 (1 H, m, aromatic H), 7.66 (1 H, m, aromatic H), 12.65 (0.6 H, s, enol OH).

Ethyl 3-(2,4-Difluoroanilino)-2-(2,4,5-trifluorobenzoyl)acrylate (13). A solution of **12** (8.4 g, 34.1 mmol) in triethyl orthoformate (85 mL, 51.2 mmol) and acetic anhydride (21.5 mL, 153 mmol) was heated at 125 °C for 2 h with removal of the ethyl acetate formed during the reaction. The solution was evaporated under reduced pressure to a mobile oil that was dissolved in methylene chloride (100 mL). 2,4-Difluoroaniline (3.82 mL, 37.5 mmol) was added to the solution. After 45 min, the solution was evaporated to dryness and crystallized from 10% ether in hexane solution, yielding 8 g (61%) of **13**, mp 85–87 °C. Anal. ($C_{18}H_{12}F_5NO_3$) C, H, N. NMR ($CDCl_3$): δ 1.03 (3 H, t, J = 7 Hz, ethyl CH_3), 4.13 (2 H, q, J = 7 Hz, ethyl CH_2), 7.06 (5 H, m, aromatic H), 8.45 (1 H, d, J = 12 Hz, olefinic H), 12.43 (1 H, bd, J = 12 Hz, NH).

Ethyl 1-(2,4-Difluorophenyl)-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (14). A 60% sodium hydride in oil suspension (730 mg, 18.3 mmol) was slowly added to a cold solution of **13** (6.85 g, 17.8 mmol) in tetrahydrofuran (100 mL).

The mixture was heated at 50 °C for 13 h under nitrogen atmosphere and was cooled and acetic acid (1 mL) was added. The solution was evaporated under reduced pressure to dryness. The residue was dissolved in methylene chloride (200 mL). The solution was washed with saturated sodium chloride solution and the organic portion was separated and dried. Upon evaporation to dryness, the residue was crystallized from ether and filtered, yielding 5 g (77%) of **14**, mp 204–205 °C. Anal. ($C_{18}H_{11}F_4NO_3$) C, H, N. NMR ($CDCl_3$): δ 1.30 (3 H, t, J = 7 Hz, ethyl CH_3), 4.37 (2 H, d, J = 7 Hz, ethyl CH_2), 6.73 (1 H, m, aromatic H), 7.25 (2 H, m, aromatic H), 7.70 (1 H, m, aromatic H), 8.17 (1 H, m, aromatic H), 8.02 (1 H, s, olefinic H).

1-(2,4-Difluorophenyl)-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (9). Hydrochloric acid (6 N, 25 mL) was added to a solution of **14** (4 g, 10.96 mmol) in trifluoroacetic acid (25 mL). The mixture was heated at 70 °C for 2 days. It was cooled, 200 mL of water was added, and the mixture was filtered. The solid was washed with water and dried, yielding 3.62 g (98%) of **9**, mp 240 °C. Anal. ($C_{16}H_7F_4NO_3$) C, H, N. NMR (DMSO-d_6) δ 7.44 (2 H, m, aromatic H), 7.73 (1 H, m, aromatic H), 7.91 (1 H, m, aromatic H), 8.36 (1 H, m, aromatic H), 8.98 (1 H, s, olefinic H), 14.45 (1 H, bs, OH).

(S)-3-Methyl-2,5-dioxopiperazine (19). A solution of carbobenzyloxyglycinesuccinimido ester (134 g, 430 mmol) in THF (600 mL) was added dropwise to a solution of L-alanine methyl ester hydrochloride (67.19 g, 480 mmol) and sodium bicarbonate (121 g, 1.44 mol) in water (600 mL) at 10 °C. After the addition, the reaction was allowed to warm up to room temperature and was stirred for 16 h. The solvent was removed under reduced pressure and the residue was taken up in ethyl acetate (2.5 L) and washed twice with water. The organic portion was separated and dried. Removal of the solvent yielded 114.1 g (90%) of **17**, mp 91–93 °C. A solution of **17** obtained from above in 1 L of methanol was hydrogenated under hydrogen atmosphere at 4 atm pressure with 10% palladium on carbon for 18 h. The solution was then filtered and the residue was washed in 3 L of methanol. The combined solution was filtered through a 0.45-μm nylon filter to remove the residual catalyst. Concentration of the filtrate to dryness yielded a solid which was digested with 1 L of boiling ethanol. It was cooled and filtered, yielding 41.17 g (83%) of **19**, mp 246–247 °C. Anal. ($C_5H_8N_2O_2$) C, H, N. NMR (D_2O): δ 1.47 (3 H, d, J = 6.5 Hz, CH_3), 4.05 (2 H, dq, J = 17 Hz, CH_2), 4.16 (1 H, m, CH). $[\alpha]^{25}_D$ = −3.78 (H_2O).

By use of this procedure, reacting D-alanine methyl ester hydrochloride instead of L-alanine methyl ester hydrochloride with carbobenzyloxyglycinesuccinimido ester, (*R*)-3-methyl-2,5-dioxopiperazine (**22**) was obtained in 85% yield, mp 247.5–249.5 °C. $[\alpha]^{25}_D$ = +3.56 (H_2O). Anal. ($C_5H_8N_2O_2$) C, H, N. NMR (D_2O): δ 1.47 (3 H, d, J = 6.5 Hz, CH_3), 4.06 (2 H, dq, J = 17 Hz, CH_2), 4.16 (1 H, m, CH).

(S)-2-Methylpiperazine Dihydrochloride (15a). (*S*)-3-Methyl-2,5-dioxopiperazine (**19**) (41.17 g, 320 mmol) was suspended in THF (1 L). A 1.35-L sample of 1 M borane–tetrahydrofuran complex (1.35 mol) was then added dropwise to the above stirring suspension over 1 h. Gas evaluation occurred and the mixture became nearly solution at the end of the addition. The mixture was refluxed for 16 h. It was cooled to room temperature and 200 mL of methanol was added over a 1-h period. Gas evolution occurred and the reaction mixture was concentrated to dryness. The residue was redissolved in methanol (500 mL) and excess methanolic HCl solution was added and the solution was refluxed for 2 h. It was cooled and allowed to stand at room temperature overnight. The precipitate was filtered to yield 29.61 g (54%) **15a**, mp >300 °C. $[\alpha]^{25.5}_D$ = −2.73 (H_2O). Anal. ($C_5H_{12}N_2 \cdot 2HCl \cdot 0.4H_2O$) C, H, N. NMR ($D_2O$): δ 1.49 (3 H, d, J = 6.5 Hz, CH_3), 3.27 (1, m, CH), 3.47 (2 H, m, NCH_2), 3.78 (4 H, m, $2NCH_2$).

By use of this procedure, (*R*)-3-methyl-2,5-dioxopiperazine (**22**) gave (*R*)-2-methylpiperazine dihydrochloride (**15b**) in 43% yield, mp >300 °C. $[\alpha]^{26}_D$ = +2.63 (H_2O). Anal. ($C_5H_{12}N_2 \cdot 2HCl$) C, H, N. NMR (D_2O): δ 1.43 (3 H, d, J = 6.5 Hz, CH_3), 3.24 (1 H, m, CH), 3.44 (2 H, m, NCH_2), 3.74 (4 H, m, $2NCH_2$).

1-(2,4-Difluorophenyl)-6-fluoro-7-((S)-3-methylpiperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid Hydrochloride (16a, (S)-Temafloxacin Hydrochloride). (*S*)-(−)-2-Methylpiperazine dihydrochloride (**19**) (25.62 g, 148 mmol) was added to a mixture of **9** (25 g, 74 mmol) and triethylamine (37.44 g, 370 mmol) in pyridine (500 mL). The reaction mixture was heated at 70 °C with stirring for 18 h. The reaction was cooled to room temperature and concentrated under reduced pressure to dryness. Ethanol was added to digest it at boiling for 30 min. The mixture was cooled and filtered, yielding 19.84 g of a solid. This solid was dissolved in 700 mL of hot dilute hydrochloric acid. A small amount of insoluble impurities was filtered off and the solution was evaporated to dryness. The residue was crystallized from ethanol–water to yield 16.35 g (49%) of **16a**, mp >300 °C. Anal. ($C_{21}H_{18}F_3N_3O_3 \cdot HCl \cdot H_2O$) C, H, N. $[\alpha]^{27.5}_D$ = −10.33 (H_2O). NMR (DMSO-d_6): δ 1.26 (3 H, d, J = 7 Hz, CH_3), 2.95 (1 H, m, NCH), 3.11 (2 H, m, NCH_2), 3.48 (4 H, m, $2NCH_2$), 6.41 (1 H, d, J_{H-F} = 7 Hz, aromatic H), 7.47 (1 H, m, aromatic H), 7.70 (1 H, m, aromatic H), 7.95 (1 H, m, aromatic H), 8.04 (1 H, d, J_{H-F} = 12 Hz, aromatic H), 9.54 (1 H, bs, NH).

By use of this procedure, 1-(2,4-difluorophenyl)-6-fluoro-7-((*R*)-3-methylpiperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride (**16b**, (*R*)-temafloxacin hydrochloride was prepared from (*R*)-2-methylpiperazine dihydrochloride (**22**) in 42% yield, mp >300 °C. Anal. ($C_{21}H_{18}F_3N_3O_3 \cdot HCl \cdot H_2O$) C, H, N. $[\alpha]^{27.5}_D$ = +11.33 (H_2O). NMR (DMSO-d_6): δ 1.26 (3 H, dd, J = 7 Hz, CH_3), 2.97 (1 H, m, NCH), 3.11 (2 H, m, NCH_2), 3.46 (4 H, m, $2NCH_2$), 6.40 (1 H, d, J_{H-F} = 7 Hz, aromatic H), 7.46 (1 H, m, aromatic H), 7.73 (1 H, m, aromatic H), 7.95 (1 H, m, aromatic H), 8.03 (1 H, d. J_{H-F} = 12 Hz, aromatic H), 8.85 (1 H, s, NH).

In Vitro Antibacterial Activity. The in vitro antibacterial activity of the test compounds was tested in a side-by-side comparison with temafloxacin and determined by conventional agar dilution procedures. The organisms were grown overnight in brain–heart infusion (BHI) broth (Difco 0037-01-6) at 36 °C. Two-fold dilutions of the stock solution (2000 μg/mL) of the test compound were made in BHI agar to obtain the test concentration ranging from 200 to 0.005 μg/mL. The plate was inoculated with approximately 10^4 organisms. It was then incubated at 36 °C for 18 h. The minimal inhibitory concentration (MIC) was the lowest concentration of the test compound that yielded no visible growth on the plate.

In Vivo Antibacterial Activity. The in vivo antibacterial activity of the test compounds was determined in CF-1 female mice weighing approximately 20 g. Aqueous solutions of the test compounds were made by dissolving the hydrochloride salt in distilled water and diluting it with distilled water to the desired volume. The median lethal dose of the test organism was determined as follows.

After 18-h incubation, the cultures of test organism in BHI broth were serially diluted by using 10-fold dilutions in 5% (w/v) hog gastric mucin. Cultures (0.5 mL), dilution from 10^{-1} to 10^{-8}, were injected intraperitoneally into mice. The LD_{50} for the test organism was calculated from cumulative mortalities on the sixth day by using the Reed and Muench procedure.[22]

After 18 h culture of the above was diluted in 5% (w/v) hog gastric mucin to obtain 100 times the LD_{50}, and 0.5 mL was injected intraperitoneally into mice. The mice were treated subcutaneously (sc) or orally (po) with a specific amount of the test compound divided equally to be administered at 1 and 5 h after infection. A group of 10 animals each for at least three dose levels was thus treated, and the deaths were recorded daily for 6 days. Ten mice were left untreated as infection control. ED_{50} values were calculated from the cumulative mortalities on the sixth day after infection by using the trimmed version of the logit method.[23]

Solubility Studies. The solubility studies were run using excess drug on a fixed volume of distilled water or in 0.05 M, pH 7.5 potassium phosphate buffer at ambient temperature. Samples were filtered and the filtrates were assayed by UV. A Hewlett-Packard 8452 diode array spectrophotometer equipped with a

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Chemstation was used for all UV spectrophotometric measurements. Complete spectra were quantitatively compared to known standards with use of a large segment of the spectra.

DNA Gyrase Inhibitor Activity. The DNA holoenzyme was prepared according to the procedure described[24] using a heparin-sepharose affinity column. The DNA gyrase supercoiling activity was assayed by a gel electrophoresis technique.[25] A 1% agarose horizontal gel slab was used. The amount of relaxed plasmid (CoE1) band and the supercoil band formed was determined by tracing the photographic negatives of the gel on a LKB Model 2022 Ultroscan densitometer. Because of the noncompetitive nature of the inhibitors, K_i may be determined as the concentration that caused 50% inhibition of the supercoil band formation.

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Synthesis and Biological Evaluation of Dipeptidyl and Tripeptidyl Polyoxin and Nikkomycin Analogues as Anticandidal Prodrugs

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Nine analogues (**1–5**, **9–12**) of the peptidyl nucleoside antibiotics nikkomycin and polyoxin were synthesized and tested for their biological properties against different strains of the pathogenic yeast *Candida albicans*. The tripeptidyl series of analogues (**1–5**) was designed to behave as prodrugs, releasing a toxic moiety upon enzymatic hydrolysis inside the cell. The dipeptidyl series (**9–12**) was designed as double-targeted drugs, being themselves toxic and releasing a toxic amino acid upon hydrolysis. All the analogues were prepared by coupling suitably protected amino acid *p*-nitrophenyl esters to 1-(5′-amino-5′-deoxy-α-D-allofuranuronosyl)uracil (UPOC) or the corresponding polyoxins and nikkomycins, with subsequent removal of the protecting group. Improved coupling yields were observed when DMSO was used as the solvent. Products were purified with use of reversed-phase HPLC and, in one case, diastereomeric products (compound **11**) were resolved by using this procedure. One of the tripeptidyl nikkomycins behaved as a prodrug but none of the compounds, as measured by in vitro testing, proved more effective than nikkomycin as an anticandidal agent.

Introduction

Opportunistic infections by *Candida albicans* are major contributors to morbidity and mortality in immunocompromised hosts.[1,2] Since the drugs currently in use for the treatment of candidiasis suffer from significant clinical limitations, a clear need exists for the development of effective anticandidal drugs.

Polyoxins and nikkomycins, closely related families of peptidyl nucleoside antibiotics, produced by species of *Streptomyces*, are potent competitive inhibitors of *C. albicans* chitin synthetase.[3,4] However, these compounds are not very effective fungicidal agents, when measuring growth of *C. albicans* in culture. These findings could be the result of the failure of these antibiotics to accumulate intracellularly or to their metabolism by the yeast. Degradation inside the cell does not seem to be the problem, since polyoxins have been shown to resist *Candida* peptidases.[5] In contrast, polyoxin and nikkomycin permeation into the cell takes places through peptide permeases; this is the step that appears to be rate limiting in the case of *C. albicans*.[3,6]

To explore increasing the uptake of peptidyl nucleosides by *C. albicans*, we have prepared a number of tripeptidyl nikkomycins and polyoxins using amino acid residues expected to improve recognition by the tripeptide transport system (Table I). These tripeptide prodrugs should not be inhibitors of chitin synthetase but should be hydrolyzed to toxic dipeptides. We have also synthesized a variety of polyoxin analogues containing a known antimetabolite (Table II, **10–12**). These multitargeted drugs have the potential to inhibit chitin synthetase and release the toxic amino acids oxalysine,[7] *m*-fluorophenylalanine[8] and N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP).[9] In this communication we report the synthesis of the above analogues and the evaluation of their stability and anticandidal activity.

Chemistry

1-(5′-amino-5′-deoxy-α-D-allofuranuronosyl)uracil, which we have previously designated UPOC (uracil polyoxin C),[5] is the carboxyterminus amino acid of the synthetic dipeptides **9**, **10**, **11**, **12**, and of tripeptides **1** and **2**. It was synthesized from uridine, according to Damodaran et al.[10]

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