appropriate fractions were combined and spin evaporated in vacuo. The residue was recrystallized from ethanol to give 0.61 g (55%) of **45**: mp 157–160 °C; UV (pH 7 buffer + 9.5% EtOH) λ_{max} 279 (ϵ 21 300); NMR (Me_2SO-d_6) δ 8.18 (s, 1 H, purine H), 6.29–7.16 (complex m, 4 H, Ar), 5.19 (s, 2 H, CH_2), 5.08 (br s, 2 H, NH_2), 3.45 (br s, 6 H, NMe_2); MS, *m/e* 302 (M^+), 273 ($M^+ - 29$), 196 ($M^+ - C_7H_8N$), 106 ($C_7H_8N^+$).

Method D. 9-Benzyl-6-(dimethylamino)-9*H*-purine (3). A mixture of **27** (0.900 g, 3.13 mmol), sodium acetate (0.41 g, 5.0 mmol), absolute ethanol (200 mL), and 5% Pd/C (0.090 g) was shaken in the presence of hydrogen at 2–3 atm for 6.5 h. The reaction mixture was left idle for 18 h, then filtered through Celite (Preiser Scientific, Inc.), and concentrated under reduced pressure. The residue was recrystallized from heptane to give 0.53 g (67%) of **3**, mp 126–127 °C, which was identical with a sample prepared from 6-chloropurine by mixture melting point, TLC, UV, and NMR.[14,15]

7-Benzyl-2-chloro-6-(dimethylamino)-7*H*-purine (52). A mixture of 7-benzyl-2,6-dichloro-7*H*-purine[12] (1.00 g, 3.58 mmol) and ethanolic dimethylamine (2.2 M, 100 mL) was warmed to dissolve the solids and then stirred at ambient temperature for 18 h. The resultant mixture was concentrated under reduced pressure to near dryness, and the solids were dispersed in ethyl acetate. The dimethylamine hydrochloride salt was removed by filtration, and the filtrate was concentrated to an oil. The oil was dissolved in toluene and filtered, and the filtrate was diluted with petroleum ether (bp 30–60 °C). Cooling on ice caused a solid to form, mp 122–126 °C. The solids were redissolved in toluene and washed with water, and the organic phase was dried over calcium chloride. The mixture was filtered, and the filtrate was concentrated to a solid residue. The solid was recrystallized from toluene–petroleum ether (bp 30–60 °C) to give 0.70 g (68%) of **52**, mp 128–130 °C. To remove a fluorescent impurity, the solids were dissolved in dichloromethane, and the solution was passed through Super Filtrol No. 19. The filtrate was reduced to dryness, and the solid residue was recrystallized from toluene–petroleum ether (bp 30–60 °C) to give an analytically pure material: mp 130–31 °C; UV (pH 7 buffer + 9.5% EtOH) λ_{max} 296.5 (ϵ 13 100); NMR (Me_2SO-d_6) δ 8.20 (s, 1 H, purine H), 6.97–7.47 (complex m, 5 H, Ar), 5.61 (s, 2 H, CH_2), 3.03 (s, 6 H, NMe_2); MS, *m/e* 287 (M^+), 258 ($M^+ - 29$), 196 ($M^+ - C_7H_7$), 91 ($C_7H_7^+$). Anal. ($C_{14}H_{14}ClN_5$) C, H, N.

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Registry No. 1, 115204-49-4; 2, 115204-50-7; 3, 6332-42-9; 4, 112089-03-9; 5, 112089-04-0; 6, 112089-05-1; 7, 112089-06-2; 8, 13233-85-7; 9, 112089-07-3; 10, 112089-08-4; 11, 112089-09-5; 12, 115204-51-8; 13, 112089-10-8; 14, 112089-11-9; 15, 112089-12-0; 16, 112089-13-1; 17, 112089-14-2; 18, 112089-15-3; 19, 112089-16-4; 20, 112089-17-5; 21, 112089-18-6; 22, 112089-19-7; 23, 7008-56-2; 24, 112089-20-0; 25, 115204-52-9; 26, 112089-22-2; 27, 115204-53-0; 28, 115204-54-1; 29, 115204-55-2; 30, 115204-56-3; 31, 115204-57-4; 32, 115204-58-5; 33, 115204-59-6; 34, 115204-60-9; 35, 115204-61-0; 36, 115204-62-1; 37, 115204-63-2; 38, 115204-64-3; 39, 115204-65-4; 40, 115204-66-5; 41, 115204-67-6; 42, 115204-68-7; 43, 115204-69-8; 44, 115204-70-1; 45, 115204-71-2; 46, 79064-26-9; 47, 115204-72-3; 48, 115204-73-4; 49, 115204-74-5; 50, 115204-75-6; 51, 115204-76-7; 52, 115204-77-8; I, 5451-40-1; II (R = 4-Et), 115204-78-9; II (R = 4-Pr-*i*), 115204-79-0; II (R = 4-*t*-Bu), 115204-80-3; II (R = O-I), 115204-81-4; II (R = 3-Cl), 115204-82-5; II (R = 3,4-Cl_2), 115204-83-6; II (R = 3-Me), 115204-84-7; II (R = 3-OMe), 115204-85-8; II (R = 3-NO_2), 115204-86-9; II (R = 3-OCH_2Ph), 115204-87-0; V (R = H), 56025-87-7; *p*-$ClC_6H_4CH_2Br$, 622-95-7; *p*-$MeC_6H_4CH_2Br$, 104-81-4; *p*-$MeOC_6H_4CH_2Br$, 2746-25-0; *p*-$O_2NC_6H_4CH_2Br$, 100-11-8; *p*-$NCC_6H_4CH_2Br$, 17201-43-3; benzyl bromide, 100-39-0; 9-(2-naphthylmethyl)-2,6-dichloro-9*H*-purine, 115204-88-1.

New "Ofloxacin" Type Antibacterial Agents. Incorporation of the Spiro Cyclopropyl Group at N-1

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The first example incorporating a spiro cyclopropyl group into an "ofloxacin" type of quinolone antibacterial agent has been prepared by potassium fluoride mediated ring closure of the hydroxymethyl cyclopropyl intermediate to give 9′-fluoro-7′-oxo-10′-(1-piperazinyl)spiro[cyclopropane-1,3′(2′*H*)-[7*H*]pyrido[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylic acid. Analogues were made by substitution at C-7 by various complex amines. Evaluation of these compounds for antibacterial activity was carried out. All examples prepared and examined showed in vitro minimum inhibitory values and in vivo mouse protection results to be diminished as compared to the parent, ofloxacin.

Within the "quinolone" class of antiinfective agents, there exists a series of potent tricyclic compounds containing a three-atom bridge connecting the quinolone N-1 and C-8 positions. This series is typified by ofloxacin[1-4] (**1a**), flumequine, and methylflumequine[5] (**1b,c**) (Figure 1).

Various modifications of this class of compounds have been reported. The information available suggests that the benzoxazine quinolone structural framework (**1**), formally 7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, is optimal relative to the carbon[5] or sulfur[6]

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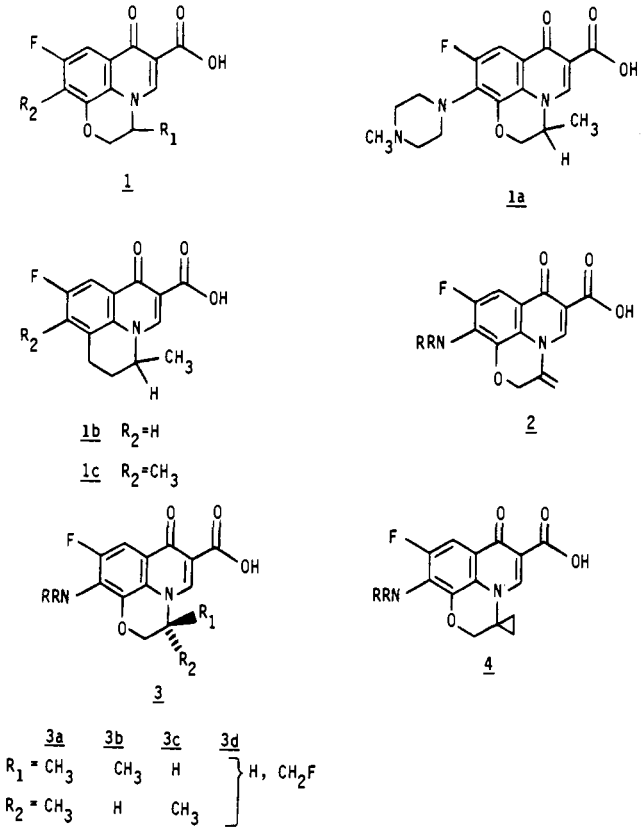

Figure 1.

analogues. Further modification of this structure at the carbon attached to the N-1 position gives an interesting series of analogs of **1**. These modifications include exomethylene[7] (**2**), dimethyl[8] (**3a**), the resolved monomethyl derivatives[9,10] (**3b,c**), and the fluoromethyl[11] compound (**3d**).

In our ongoing program on quinolone antiinfectives, we have sought to prepare new and more potent compounds of this class. This led us to consider the possibility of combining the features of the tricyclic ring structure **1** with the N-1 cyclopropyl moiety, found in ciprofloxacin,[12] to produce a compound such as **4**.

Replacement of the ofloxacin methyl group by a spiro cyclopropane was envisioned as having three potentially useful features. The cyclopropyl moeity at N-1 has been shown to improve in vitro and in vivo potency substantially,[13] would remove chirality from this type of structure, and would be less sterically demanding than the inactive dimethyl analogue[8] **3a**.

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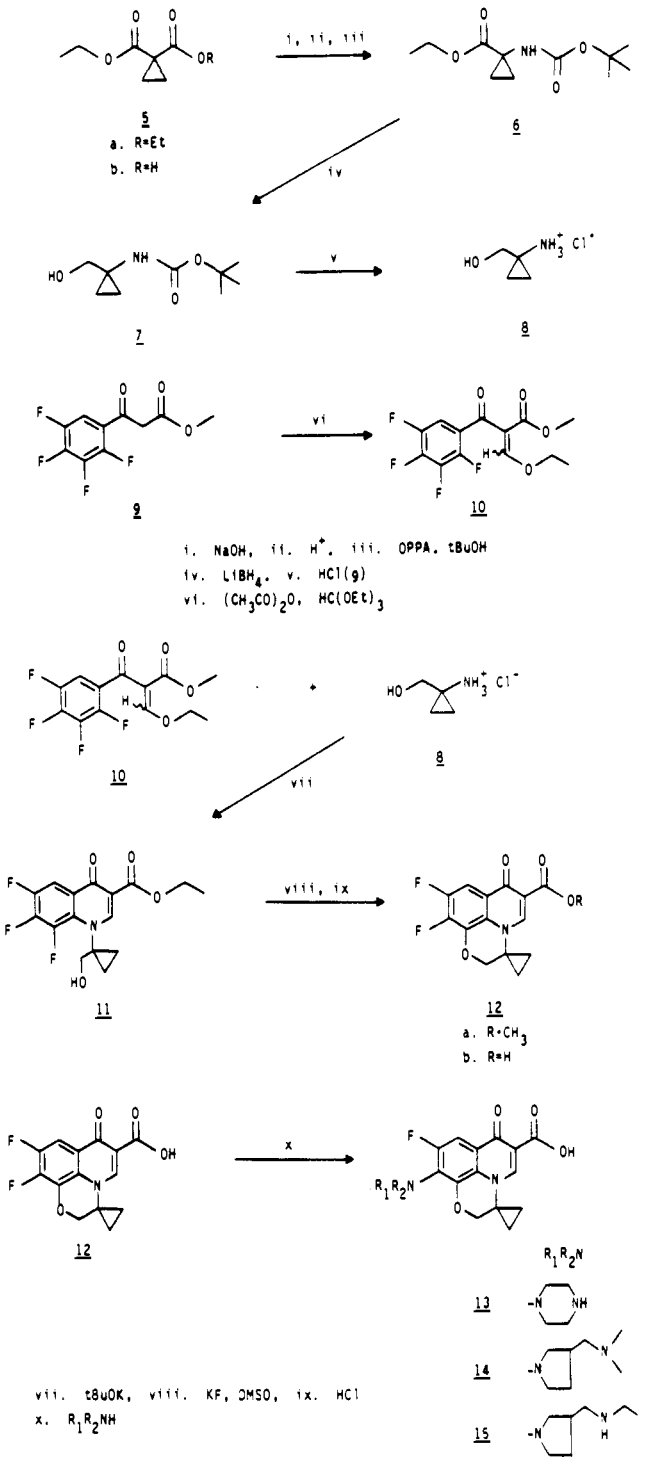
Scheme I



We report here the synthesis of this type of compound and its microbiological potency. After the completion of this work but prior to the submission of this paper, a European patent (EP0206076, 0206101) from the Bayer Co. was published claiming compounds of this type. However, no supporting synthetic or biological data were provided.

Chemistry

Scheme I shows the synthesis of the target compounds. Starting from 1,1-cyclopropanedicarboxylic acid diethyl ester (**5a**), hydrolysis and in situ formation/rearrangement of the intermediate acylazide in the presence of *tert*-butyl alcohol gave the *N*-Boc-1-amino-1-carbethoxycyclopropane[14] (**6**). Reduction of **6** with lithium borohydride

Table I. Biological Testing Results Minimum Inhibitory Concentrations[a] (μg/mL)

	GYR, I_{50}[b]	*E. coli* H560	*Entero. cloacae*	*E. coli vogel*	*Klebs. pneumonia*	*Proteus rettgeri*	*Pseudomonas aeruginosa*	*Staph. aureus* Resist	*Staph. aureus* Sens.	*Strep. faecalis*	*Strep. pneumonia*	*Strep. pyogeneous*	in vivo[c] PD_{50}, mg/kg *E. coli vogel* po	sc
1a[d]	2.25	0.05	0.1	0.1	0.1	0.2	0.4	0.4	0.1	0.8	0.8	0.8	2.0	0.8
							Ofloxacin Analogues							
16	3.00	0.1	0.1	0.2	0.2	0.4	1.6	3.1	0.4	6.3	3.1	0.4	NT	
17	NT[e]	0.05	0.2	0.1	0.4	0.4	0.8	0.2	0.006	0.05	0.025	0.006	>100	11
18	3.00	0.2	0.4	0.2	0.4	0.8	3.1	0.2	0.05	0.4	0.05	0.05	6.2	2.6
							Spirocyclopropyl Containing Compounds							
13	11.25	0.4	0.4	0.8	0.8	1.6	0.8	6.3	1.6	6.3	12.5	12.5	NT	
14	11.25	0.8	0.8	0.4	0.8	3.1	6.3	0.8	0.2	0.8	0.4	0.4	16	6.3
15	5.5	1.6	0.8	0.8	3.1	3.1	6.3	1.6	0.1	0.8	0.05	0.05	NT	

[a] Standard microdilution techniques; see ref 18. [b] Minimum concentration of drug needed to produce linear DNA at an intensity relative to oxolinic acid at 10 μg/mL. [c] Standard mouse protection techniques, see ref 18. [d] Ofloxacin. [e] Not tested.

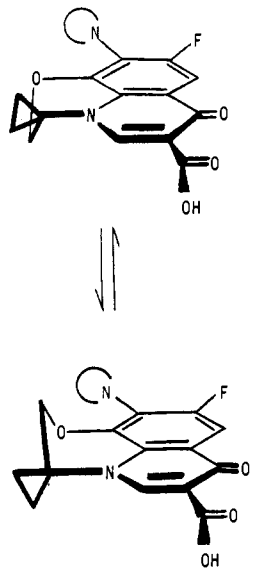


Figure 2. "C" ring inversion, movement of cyclopropane ring.

afforded the *N*-Boc-1-amino-1-(hydroxymethyl)cyclopropane (**7**). Removal of the "Boc" protecting group with gaseous HCl gave the amine hydrochloride (**8**). Reaction of the β-keto ester[15] (**9**) with triethylorthoformate/acetic anhydride provided α-(ethoxymethylene)-β-keto ester[10] (**10**), which was used without isolation in the next reaction. Condensation of **8** with **10** and subsequent cyclization yielded the 1-[(1′-hydroxymethyl)cyclopropyl]-4-oxo-3-quinolonecarboxylic acid ethyl ester (**11**). This compound was cyclized to the desired tricyclic benzoxaine (**12a**) through the action of KF in DMSO at 100 °C[16] followed by hydrolysis of the carboxylic acid ester. Coupling of **12b** with the requisite amines gave the target compounds (**13–15**).

Microbiology

Determination of the inhibition of the gyrase enzyme (I_{50}, *Escherichia coli* H560) was done by the method of Domagala et al.[17] The minimum inhibitory concentrations (MIC) and protective doses in mice (PD_{50}) against a challenge of *E. coli Vogel* were performed according to the method of Heifetz et al.[18] The results of the determinations are presented in Table I.

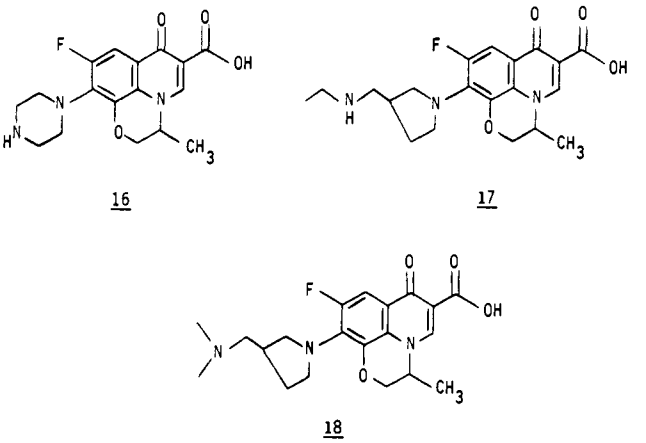


Figure 3.

Results and Discussion

In structures **13–15**, the consequence of having a cyclopropane ring attached to the benzoxazine in a spiro fashion is to fix the plane of the cyclopropane perpendicular to the plane of the benzoxazine rings. There is still some mobility of the cyclopropane ring however. As the "C" ring of the benzoxazine inverts, the cyclopropane moiety moves up and down in a plane perpendicular to the quinolone ring plane (Figure 2). This restricted movement may have implication regarding the activity, and it is clear from the data obtained (Table I) that the addition of the cyclopropane to this structure has not imparted the expected positive effect on either gyrase inhibition or MIC values when compared to the corresponding ofloxacin derivatives **16–18**[19] (Figure 3).

For ofloxacin[1,2] and methylflumequine,[5] it has been determined that only the *S*-(−) enantiomer having the methyl group above the quinolone ring plane possesses activity. The enantiomeric *R*-(+) isomer, with the methyl group down, is significantly less active. The dimethyl analogue is inactive. The decreased or lack of activity of these compounds may be attributable to some steric interaction caused by the projection of a methyl group below the plane of the rings. Perhaps the current compounds have enought bulk from the cyclopropane methylene

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projecting below the benzoxazine plane that the cyclopropane's contribution to increased activity is negated by an unfavorable steric interaction. Thus the conformationally restricted cyclopropane ring in the present case represents some intermediate point between the favorable cyclopropane contribution to ciprofloxacin and the unfavorable interactions of a dimethylated benzoxazine structure.

Experimental Section

Melting points were taken on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Nicolet FT IR SX-20 with 2-cm^{-1} resolution. Proton magnetic resonance (^{1}H NMR) were recorded on a Varian XL-200 spectrometer. Chemical shifts are reported in δ units relative to tetramethylsilane. Mass spectra were recorded on either a Finnigan 4500 GCMS or a VG Analytical 707OE/HF instrument with an 11/250 data system. Column chromatography was performed with W. R. Grace silica gel 60, 230–400. Solutions were dried over magnesium sulfate. All concentrations of solutions were performed under reduced pressure. C, H, N elemental analyses were performed on either a Control Equipment Corp. Model 240XA or a Carlo-Erba Model 1106 elemental analyzer and halogen determinations were performed by the closed flask combustion method, employing a titrimetric determination. All new products and intermediates had analytical results within ±0.4% of theoretical values. HPLC purity of the final products was performed on reverse-phase C18 columns with 20% THF/80% 0.05M ammonium phosphate buffer (pH 3.0) mobile phase at 1.0 mL/min with product detection by absorbance at 287 nm.

1,1-Cyclopropanedicarboxylic Acid Monoethyl Ester (5b). Sodium hydroxide (1 N, 532 mL) was added to a solution of 1,1-cyclopropanedicarboxylic acid diethyl ester[20] (**5a**) (99.1 g, 0.53 mol) in 95% ethanol (850 mL). The reaction mixture was stirred at room temperature overnight and then concentrated under reduced pressure to a white solid. The white residue was partitioned between H_2O/Et_2O, and the resulting phases were separated. The aqueous phase was extracted several times with Et_2O and then acidified to pH with 6 N HCl. The acidified aqueous layer was extracted four times with Et_2O. The organics were combined, dried, and concentrated to give **5b** as a slightly yellow oil: 64.8 g (77%); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.1 Hz, 3 H), 1.63–1.87 (m, 4 H), 4.22 (q, J = 7.1 Hz, 2 H); IR (LF) 2996, 1767, 1729 cm^{-1}; MS, m/z 159 (M^+ + 1), 141 (base). Anal. ($C_7H_{10}O_4$) C, H.

1-[[(1,1-Dimethylethoxy)carbonyl]amino]cyclopropanecarboxylic Acid Ethyl Ester (6). To **5b** (63.9 g, 0.40 mol) in *tert*-butyl alcohol (80 mL) was added Et_3N (43.8 g, 0.43 mol) and diphenyl phosphorazidate (122 g, 0.44 mol). The reaction mixture was heated to reflux. After 5 h, the reaction mixture was cooled to room temperature and the volatiles were removed. The residue was suspended in EtOAc (100 mL) and washed with 5% citric acid solution (3 × 33 mL), saturated $NaHCO_3$ (3 × 33 mL), and saturated NaCl (3 × 15 mL), respectively. The organic layer was dried and concentrated giving **6** as a pale yellow oil: 6.22 g (83%); ^{1}H NMR ($CDCl_3$) δ 1.05–1.30 (m, 5 H), 1.37–1.57 (m, 11 H), 4.11 (q, J = 7.2 Hz, 2 H) 5.20 (br s, 1 H); IR (LF) 3368, 2980, 1728 cm^{-1}; MS, m/z 230 (M^+ + 1), 57 (base). Anal. ($C_{11}H_{19}NO_4 \cdot 0.3H_2O$) C, H, N.

[1-(Hydroxymethyl)cyclopropyl]carbamic Acid 1,1-Dimethylethyl Ester (7). While under a N_2 atmosphere, $LiBH_4$ (8.00 g, 0.37 mol) was weighed into a three-neck flask and suspended in Et_2O (200 mL). To this mixture was added dropwise a solution of **6** (55.7 g, 0.24 mol) in Et_2O (200 mL). The reaction mixture was stirred at room temperature for 2 h and then quenched by slow addition of 200 mL of MeOH (vigorous gas evolution). After being stirred overnight, the reaction mixture was poured into an equal volume of saturated NH_4Cl. The Et_2O layer was separated, and the aqueous layer was extracted four times with Et_2O. The combined Et_2O layers were dried and concentrated to a white solid (48.6 g). This material was recrystallized from diisopropyl ether to give **7** as colorless crystals: 35.4 g (78.8%); mp 82.5–84.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 4 H), 1.43 (s, 9 H), 3.57 (s, 2 H), 5.18 (s, 1 H); IR (KBr) 3345, 3274, 2988, 2925, 1695 cm^{-1}; MS, m/z 188 (M^+ + 1), 57 (base). Anal. ($C_9H_{17}NO_3$) C, H, N.

1-Aminocyclopropanemethanol Monohydrochloride (8). Gaseous HCl was bubbled into a solution of 7 (15.8 g, 84.4 mmol) in CH_2Cl_2 (200 mL) for 10 min, and the cloudy solution was stirred for 1 h at room temperature. The resulting solids were collected by filtration to give 8 as colorless crystals: 4.52 g (43%); mp 119 °C dec; ^{1}H NMR (DMSO) δ 0.66–0.80 (m, 2 H), 0.82–0.97 (m, 2 H), 3.47 (d, J = 5.4 Hz, 2 H), 5.30 (t, J = 5.4 Hz, 1 H), 8.30 (br s, 3 H); IR (KBr) 3341, 2941, 2033 cm^{-1}; MS, m/z 87 (M^+), 42 (base). Anal. ($C_4H_{10}ClNO$) C, H, N.

The filtrate from above was concentrated, and the residue was resuspended in CH_2Cl_2 (250 mL). HCl gas was bubbled through the solution for 20 min. The reaction mixture was stirred for 2 h at room temperature, and the resulting solid was filtered off to give 8 as colorless crystals: 4.25 g (40%); mp 117–119 °C; ^{1}H NMR and IR analyses were identical with those reported above; MS, m/z 87 (M^+), 56 (base). Anal. ($C_4H_{10}ClNO$) C, H, N.

6,7,8-Trifluoro-1,4-dihydro-1-[1-(hydroxymethyl)cyclopropyl]-4-oxo-3-quinolinecarboxylic Acid Methyl Ester (11). 2,3,4,5-Tetrafluoro-β-oxobenzenepropanoic acid methyl ester (**9**) (4.05 g, 16.2 mmol), acetic anhydride (3.97 g, 38.8 mmol), and triethyl orthoformate (3.60 g, 24.3 mmol) were combined and heated to reflux (oil bath temperature, 155 °C). The reaction mixture was refluxed for 2.5 h and then cooled to 80 °C. The volatiles were removed (0.1 mm) at 80 °C followed by cooling the reaction to 45 °C. A suspension of 8 (2.00 g, 16.2 mmol) and potassium *tert*-butoxide (1.82 g, 16.2 mmol) in *tert*-butyl alcohol (85 mL) was added to the residue, and the reaction mixture was stirred at 45 °C for 3 h followed by addition of a second equivalent of potassium *tert*-butoxide (1.82 g, 16.2 mmol). The reaction mixture was stirred overnight at 45 °C and then cooled to room temperature. Unreacted potassium *tert*-butoxide was destroyed by addition of 1–2 mL of acetic acid. The reaction mixture was concentrated. The resulting orange solid was suspended in water and filtered. The resulting filter pad was dissolved in hot $CHCl_3$ (excess H_2O removed by separation). Crystallization gave **11** as a light yellow powder: 2.68 g (51%); mp 273.5–276 °C; ^{1}H NMR (DMSO-d_6) δ 1.17–162 (m, 4 H), 3.25 (d, J = 12.1 Hz, 1 H), 3.77 (s, 3 H), 4.10–4.23 (m, 1 H), 5.23 (br s, 1 H), 7.90–8.08 (m, 1 H), 8.60 (s, 1 H); IR (KBr) 3424, 1734, 1709, 1617, 1486, 803 cm^{-1}; MS, m/z 327 (M^+) 53 (base). Anal. ($C_{15}H_{12}F_3NO_4 \cdot 0.65H_2O$) C, H, N.

9′,10′-Difluoro-7′-oxospiro[cyclopropane-1,3′(2′*H*)-[7*H*]-pyrido[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylic Acid (12b). Potassium fluoride (dried at >350 °C for 2 days) (0.33 g, 5.68 mmol) and **11** (0.50 g, 1.53 mmol) in dry DMSO (5mL) was heated to 115 °C. After being stirred overnight, the reaction mixture was cooled to room temperature and the solvent was removed. The residual solid was triturated with H_2O and filtered. The filter pad was washed several times with H_2O followed by washing with Et_2O until dry, giving 0.53 g of crude **12a**. The crude **12a** was suspended in 3 N HCl (3 mL) and glacial acetic acid (4 mL) and then heated to reflux. The reaction became homogeneous, and then a precipitate formed. After the mixture was stirred at reflux for 2.5 h, the heat was removed and the suspension was filtered. The filter pad was washed with water three times and then with Et_2O until dry. The crude yield of **12b** was 0.25 g (56%). A portion of **12b** was recrystallized from DMSO, giving a white solid: yield 0.11 g; mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 1.13–1.50 (m, 2 H), 1.67–2.00 (m, 2 H), 4.59 (s, 2 H), 7.77–8.00 (m, 1 H), 8.55 (s, 1 H), 14.78 (s, 1 H); IR (KBr) 3443, 1711, 1624, 1571, 1486, 1075, 808 cm^{-1}; MS, m/z 293 (M^+), 249 ($M^+ - CO_2$, base). Anal. ($C_{14}H_9F_2NO_4 \cdot 0.5H_2O$) C, H, N, F.

General Method of Coupling. By use of established procedures,[16] 9′10′-difluoro-7′-oxospiro[cyclopropane-1,3′(2′*H*)-[7*H*]-pyrido[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylic acid (**12b**) was heated in CH_3CN at reflux in the presence of the appropriate amine and a HF acceptor (Et_3N or DBU). When the reaction was complete by TLC, the reaction mixture was cooled to room temperature, and the crude produce was collected by filtration, washed with solvents, and dried under vacuum.

9′-Fluoro-7′-oxo-10′-(1-piperazinyl)spiro[cyclopropane-1,3′(2′*H*)-[7*H*]pyrido[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylic Acid (13). As described above, **12b** (0.26 g, 0.89 mmol) was

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reacted with piperazine (0.31 g, 3.60 mmol). The HF acceptor was omitted. The product was washed with Et_2O, cold CH_3OH, and Et_2O. The yield of **13** was 0.26 g (81.3%); mp 233 °C dec; ^1H NMR(TFA) δ 1.60–1.78 (m, 2 H), 1.88–2.08 (m, 2 H), 3.57–3.85 (m, 4 H), 3.90–4.10 (m, 4 H), 4.65 (s, 2 H), 8.07 (d, J = 11.4 Hz, 1 H), 8.97 (s, 1 H); IR (KBr) 3435, 1726, 1620 cm^{-1}; MS, m/z 360 (M^+ + 1, base); HPLC 99.3%. Anal. ($C_{18}H_{18}FN_3O_4 \cdot 1.13H_2O \cdot 0.14HF$) C, H, N, F.

10′-[3-[(Ethylamino)methyl]-1-pyrrolidinyl]-9′-fluoro-7′-oxospiro[cyclopropane-1,3′(2′*H*)-[7*H*]pyrido[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylic Acid (15). As described above, **12b** (0.27 g, 0.92 mmol) was reacted with *N*-ethyl-3-pyrrolidinemethanamine, (0.17 g, 1.32 mmol) and Et_3N (0.10 g, 0.99 mmol). The product was washed with CH_3CN and Et_2O to give crude **15** (0.32 g, 86%). A portion of crude **15** (0.24 g) was purified as follows: The solid was suspended in H_2O, and 1 N NaOH was added to give a solution (pH 11.6). This solution was filtered, and the pH was adjusted to 1 with 1 N HCl. The solution was lyophilized to a gum, which crystallized from hot isopropyl alcohol to give 0.20 g of pure **15**: mp 254 °C dec; ^1H NMR (TFA) δ 1.27–2.30 (m, 7 H) 2.34–2.70 (m, 1 H), 2.72–3.00 (m, 1 H), 3.23–3.93 (m, 6 H), 4.15–5.30 (m, 5 H), 7.15 (br s, 1 H), 8.23 (br s, 1 H), 9.17 (br s, 1 H); IR (KBr) 3435, 1733, 1619 (cm^{-1}); MS, m/z 402 (M^+1), 58 (base); HPLC (98.8%). Anal. ($C_{21}H_{24}FN_3O_4 \cdot 1.3HCl \cdot 1.25H_2O$) C, H, Cl; N: calcd, 8.90; found, 8.45.

10′-[3-[(Dimethylamino)methyl]-1-pyrrolidinyl]-9′-fluoro-7′-oxospiro[cyclopropane-1,3′(2′*H*)-[7*H*]pyrido-[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylic Acid (14). As described above, **12b** (0.40 g, 1.36 mmol) was reacted with *N,N*-methyl-3-pyrrolidinemethanamine (0.19 g, 1.48 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.23 g, 1.51 mmol). No solids were formed upon cooling, and the reaction mixture was evaporated to a gum. This gum was triturated with Et_2O to give a solid, which was collected by filtration, suspended in H_2O, and basified with 1 N NaOH, and the resulting solution (pH 12) was extracted four times with Et_2O. The pH of the aqueous layer was adjusted to 7 and extracted five times with $CHCl_3$. The combined $CHCl_3$ extracts were dried, filtered, and evaporated to give **14** as a solid: 0.31 g (56%); mp 193–197 °C; ^1H NMR ($CDCl_3$) δ 1.16–1.42 (m, 2 H), 1.45–1.87 (m, 3 H), 1.98–2.20 (m, 1 H), 2.22–2.67 (m, 9 H), 3.43–3.98 (m, 4 H), 4.22 (ab q, J = 11.6 Hz, 1 H), 4.26 (ab q, J = 11.6 Hz, 1 H), 7.66 (d, J = 13.9 Hz, 1 H), 8.28 (s, 1 H); IR (KBr) 3443, 1724, 1620 cm^{-1}; MS, m/z 401 (M), 58 (base); HPLC 98.7%. Anal. ($C_{21}H_{24}FN_3O_4 \cdot 0.05CHCl_3$) C, H, N, F.

Registry No. **5a**, 1559-02-0; **5b**, 3697-66-3; **6**, 107259-05-2; **7**, 107017-73-2; **8**, 115652-52-3; **9**, 111630-16-1; **10**, 115652-53-4; **11**, 113211-50-0; **12a**, 115652-51-2; **12b**, 113211-52-2; **13**, 113211-55-5; **14**, 115652-54-5; **15**, 113211-56-6; **16**, 82419-52-1; **17**, 91196-82-6; **18**, 115652-55-6; piperazine, 110-85-0; *N,N*-dimethyl-3-pyrrolidinemethanamine, 99724-17-1; *N*-ethyl-3-pyrrolidinemethanamine, 91187-83-6.

Studies on Antifungal Agents. 23. Novel Substituted 3,5-Diphenyl-3-(1*H*-imidazol-1-ylmethyl)-2-alkylisoxazolidine Derivatives

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The synthesis and antifungal activity of a novel series of substituted 3,5-diphenyl-3-(1*H*-imidazol-1-ylmethyl)-2-alkylisoxazolidine derivatives (**15–30**) are described. The synthesis of the title compounds was accomplished via a 1,3-dipolar cycloaddition reaction of α-substituted ketonitrones with appropriate styrene precursors. The compounds when tested in vitro in solid agar cultures exerted a very potent antifungal activity against a wide variety of yeast and systemic mycoses and dermatophytes, especially *Trichophyton* and *Microsporum* sp., *Epidermophyton floccosum* and *Candida stellatoidea*. The in vitro activity against *Aspergillus fumigatus* and *Candida albicans* was moderate to potent. Overall, the two bis(4-chlorophenyl) analogues **18** and **19** were the most potent in vitro compounds, showing MIC values ranging between 0.2 and 7.0 μg/mL, as compared to 0.2–20.0 μg/mL for ketoconazole, which was used as the positive standard in all assays. When tested in vivo in the rat vaginal candidiasis model, derivative **18**, although showing significant antifungal activity when compared to controls, was less effective than ketoconazole. The title 3,5-substituted isoxazolidine compounds represent a novel class of potent antifungal agents.

Over the past 2 decades or so, the frequency of systemic fungal infections in man has increased dramatically. Undoubtedly, the population most susceptible to such infections are immunocompromized patients,[1-6] especially those with hematologic malignancies (such as leukemia), acquired immune deficiency syndrome (AIDS),[7] and patients undergoing cancer chemotherapy and organ transplantation. Although *Candida* species continue to be the major patogenic fungi in immunocompromized patients, the number of other fungal infections (cryptococcosis,[8] aspergillosis,[8] zygomycosis,[8] coccidioidomycosis,[9-11] paracoccidioidomycosis,[12] and chromoblastomycosis[13]) have become increasingly worrisome.

Since its discovery in 1953,[14] and after a quarter of a century of continuing clinical use, the polyene antibiotic amphotericin B is still the drug of choice in the treatment of serious systemic fungal infections.[5,15-18] Although amphotericin B exerts an excellent activity against most pathogenic fungi, along with an impressive lack of native or developed resistance, its poor solubility in water at

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