New Antimicrobial Nitrofuran, *trans*-5-Amino-3-[2-(5-Nitro-2-Furyl)Vinyl]- Δ^2 -1,2,4-Oxadiazole: Antibacterial, Antifungal, and Antiprotozoal Activities In Vitro

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trans-5-amino-3-[2-(5-nitro-2-furyl)vinyl]- Δ^2 -1,2,4-oxadiazole, a new antimicrobial nitrofuran, has shown microbial activity in vitro against a wide range of bacteria and fungi, and against several protozoa. The antimicrobial activity of the nitrofuran is not significantly diminished in vitro in the presence of 50% human serum. The compound is not cross-resistant with a number of common antibiotics or synthetic antimicrobial agents; some cross-resistance with furazolidone is encountered. The development of resistance to the compound in vitro, when it occurs, is mixed, but is unrelated to increase in pathogenicity of the test organism.

trans-5-amino-3-[2-(5-nitro-2-furyl)vinyl]- Δ^2 -1,2,4-oxadiazole (SQ 18,506), originally synthesized by Breuer (2), possesses potent activity in vitro against gram-positive and gram-negative bacteria, certain fungi, and *Trichomonas vaginalis* (4). More recently, the compound has shown marked chemotherapeutic activity in animals infected with *Schistosoma mansoni* (3), *Schistosoma japonicum* (3), and some degree of activity against *Escherichia coli*, *Candida albicans*, or *Trichomonas foetus* (5). The present study extends the evaluation in vitro of this nitrofuran.

MATERIALS AND METHODS

Antimicrobial agents. Antimicrobial agents were supplied by Eaton Laboratories (furazolidone, lot no. 1814), Eli Lilly (penicillin V, 880 μ g/mg) and Squibb (SQ 18,506, batch 11, average particle size 3 to 5 μ m).

Antimicrobial activity. Most of the cultures used in this study were recent clinical isolates of human origin. Minimal inhibitory concentrations (MIC) of SQ 18,506 against the isolates were determined by the conventional twofold dilution method with broth or agar; the growth media and test procedures used were previously described in detail (1, 6). Minimal microbicidal concentrations (MMC) were determined by subculturing all tubes that showed no visible growth in a broth-dilution test onto antibiotic-free agar media. The MMC was defined as the lowest concentration of drug that gave no visible colonial growth on subculture after 48 h at 37 C. In experiments to determine the effect of serum on the antimicrobial activity of SQ 18,506, MIC determinations were made in the presence and absence of pooled human serum (BBL). SQ 18,506 and furazolidone were solubilized in dimethyl sulfoxide, and dilutions were prepared as

needed in distilled water before the assessment of antimicrobial activity.

Stability in solution. SQ 18,506, solubilized in dimethyl sulfoxide, and penicillin V were prepared in 0.05 M phosphate buffer (pH values 2.0 to 8.0) at a concentration of 1 mg/ml and held at 37 C for 24 h. At 4 and 24 h, 0.03-ml samples of the test solutions were assayed for bioactivity by using a disk diffusion assay on plates seeded with *Staphylococcus aureus* P-209 (1). Zones of inhibition were measured after 18 h of incubation at 37 C, and the residual activity was determined.

FIG. 1. Structure of SQ 18,506, *trans*-5-amino-3-[2-(5-nitro-2-furyl)vinyl]- Δ^2 -1,2,4-oxadiazole.

TABLE 1. Effect of human serum on antibacterial activity in vitro of SQ 18,506 and furazolidone

Organism	MIC ^a (μ g/ml)			
	SQ 18,506		Furazolidone	
	With-out serum	50% serum	With-out serum	50% serum
<i>Staphylococcus aureus</i> P-209 ...	0.09	0.63	0.33	1.25
<i>Escherichia coli</i> SC 2975	0.14	0.09	0.23	0.9

^a Minimal inhibitory concentration.

TABLE 2. Antimicrobial spectrum of SQ 18,506 *in vitro*

Organism	Squibb culture	Inoculum size ^a	SQ 18,506		Furazolidone MIC ^b (μg/ml)
			MIC ^c (μg/ml)	MMC ^c (μg/ml)	
Gram-positive bacteria					
<i>Staphylococcus aureus</i> (non-penicillinase producer)	2399		3.1		3.6
<i>S. aureus</i> (penicillinase producer)	2400		2.8	3.1	4.7
<i>Streptococcus pyogenes</i>	3862	H	0.01		0.32
<i>S. faecalis</i>	1648	H	2.4		
<i>Diplococcus pneumoniae</i>	5479	H	3.5		9.4
<i>Micrococcus lysodeikticus</i>	2529	H	4.7		
<i>Sarcina lutea</i>	2485	H	4.7		18.7
<i>Clostridium tetanomorphum</i>	3103	H	0.002		
<i>Bacillus subtilis</i>	3777	H	0.03		
<i>B. cereus</i>	3031	H	0.05		
<i>Mycobacterium tuberculosis</i>	5516		9.4		
<i>Nocardia asteroides</i>	2626		7.8		
Gram-negative bacteria					
<i>Escherichia coli</i>	2975	H	0.09	0.26	0.19
<i>E. coli</i> (O55:B5)	8516	H	0.14		0.32
<i>Proteus vulgaris</i>	9416	H	4.7		
<i>P. mirabilis</i>	3855	H	12.5		
<i>Klebsiella pneumoniae</i>	1565	H	0.05		0.6
<i>Enterobacter cloacae</i>	8236	H	3.1		
<i>Herellea vaginicola</i>	8333	H	5.5		9.4
<i>Salmonella schottmuelleri</i>	3850	H	0.02	0.06	
<i>S. gallinarum</i>	3030	H	0.1		
<i>S. typhimurium</i>	3821	H	0.05		
<i>Shigella flexneri</i>	1462	H	0.07	0.13	0.23
<i>Serratia marcescens</i>	8248	H	12.5		
<i>Pseudomonas aeruginosa</i>	8329	H	3.1		
<i>Pasteurella multocida</i>	8826	H	0.25		
<i>Haemophilus influenzae</i> ^c	8622	H	0.3		
<i>H. vaginalis</i>	8278		0.2		
<i>Neisseria gonorrhoeae</i> ^c	8270		0.05		
<i>N. catarrhalis</i>	5348		2.4		
Fungi					
<i>Aspergillus niger</i>	2828		25.0		
<i>Penicillium notatum</i>	2122		10.9		
<i>Cladosporium resinae</i>	5476		50.0		
<i>C. trichoides</i>	9006		4.7		
<i>Pullularia pullulans</i>	2599		6.3		
<i>Fusarium bulbigenum</i>	5273		18.7		
<i>Trichophyton mentagrophytes</i>	2637		12.5		
<i>T. rubrum</i>	8139		6.3		
<i>T. schoenleini</i>	8990		3.1		
<i>T. tonsurans</i>	8991		7.8		
<i>T. violaceum</i>	8995		2.3		
<i>Microsporium audouini</i>	5282		9.4		
<i>Epidermophyton floccosum</i>	8996		1.2		
<i>Phialophora jeanseimei</i>	9008		9.4		
<i>Fonsecaea pedrosoi</i>	8993		4.7		
<i>Candida albicans</i>	5314		12.5	25.0	
<i>C. krusei</i>	2616		37.5		
<i>C. tropicalis</i>	1647		25.0		
<i>Geotrichum candidum</i>	8623		3.1		

TABLE 2—Continued

Organism	Squibb culture	Inoculum size ^a	SQ 18,506		Furazolidone MIC ^b (μg/ml)
			MIC ^b (μg/ml)	MMC ^b (μg/ml)	
Parasites					
<i>Trichomonas vaginalis</i>	8560		0.78	0.78	
<i>T. foetus</i>	9644		0.6		
<i>Crithidia fasciculata</i>	8853		14.0		

^a (H) is equal to approximately 10⁶ bacteria/ml; for all other bacteria and yeasts, the inoculum was approximately 10⁸ organisms/ml; for filamentous fungi, the inoculum was 2 ml of a 1:50 dilution of a mat or spore suspension derived from a 48-h growth (26 C) of organisms on 100 ml of agar contained in a 250-ml Erlenmeyer flask.

^b MIC, Minimal inhibitory concentration; MMC, minimal microbicidal concentration.

^c Tested by agar-dilution method; for all others, the broth-dilution technique was used.

TABLE 3. Susceptibility of various clinical isolates to SQ 18,506

Organism	No. of strains	No. of isolates susceptible at MIC ^a (μg/ml)				
		<0.1	0.1-1.0	1.1-12.5	12.6-25	26-50
<i>Staphylococcus aureus</i>	10		10			
<i>Neisseria gonorrhoeae</i>	8	7	1			
<i>Haemophilus vaginalis</i>	4		1	3		
<i>Escherichia coli</i>	39		37	1	1	
<i>Herellea vaginicola</i>	5			2	3	
<i>Proteus</i> sp.	37			1	2	34
<i>Candida albicans</i>	11				9	2

^a Minimal inhibitory concentration.

Resistance development. A twofold broth-dilution test was used to ascertain the development of resistance to SQ 18,506 in vitro. After incubation for 18 h at 37 C, organisms from tubes containing the highest concentration of SQ 18,506 showing visible growth were used to inoculate the next series of tubes. The index of induced resistance was calculated at intervals, and after the final (twentieth) passage, by comparing the initial MIC of a culture to that obtained after various numbers of exposures to SQ 18,506.

Determination of median lethal dose. Experimental infections were produced in mice by the intraperitoneal injection of suitably diluted cultures. At least three different dose groups of the test organisms were used in twofold increments; each dose group consisted of ten animals. All mice were observed for fixed periods of time (*Candida*-infected, 30 to 60 h; bacteria-infected, 5 to 22 h; *Trichomonas*-infected, 7 to 14 days), and the median lethal dose was determined by the method of Reed and Muench (7).

RESULTS AND DISCUSSION

Physical and chemical properties. SQ 18,506 (Fig. 1) is a light-yellow crystalline powder that is soluble in cold dimethyl sulfoxide or hot dimethylformamide at approximately 10% (wt/vol). It is virtually insoluble in water and lower alcohols, acetone, chloroform, ether,

and benzene. Because of the low water solubility of the compound, SQ 18,506 was first solubilized in dimethyl sulfoxide before being used in studies to define its biological activity in vitro. Dilute solutions of SQ 18,506, when exposed to light, undergo rapid decomposition; concentrated solutions (about 25 mg or more per ml), allowed to stand for several days, showed less than 5% decomposition. Particle size varies from batch to batch, but can be adjusted rather easily to an average diameter of 3 to 5 μm.

When solutions of SQ 18,506 were prepared in 0.05 M phosphate buffer, pH values 2.0 and 8.0, and held at 37 C for 24 h, both SQ 18,506 and a penicillin V control showed less than 5% loss in bioactivity over the entire pH range studied. The antimicrobial activity in vitro of SQ 18,506 was not significantly diminished by the addition of 50% human serum to the medium, nor was that of furazolidone, a marketed nitrofurantoin (Table 1).

Antimicrobial activity. The exceedingly broad spectrum of SQ 18,506 was confirmed and extended to include a wide variety of bacteria and fungi. In all, 37 (77%) of 48 important disease-producing microorganisms were susceptible to the compound at an MIC of 10 μg/ml or

TABLE 4. Development of resistance to SQ 18,506 on exposure to increasing concentrations of the nitrofurans

Organism	SC no.	Initial MIC ^a (μg/ml)	Index of induced resistance ^b				Change in LD ₅₀ ^c after 20 exposures
			No. of exposures				
			5	10	15	20	
<i>Staphylococcus aureus</i>	2400	2.4	4	4	10	10	NC ^d
<i>Escherichia coli</i>	8517	0.3	5	15	63	63	NC
<i>Salmonella schottmuelleri</i>	3850	0.05	4	4	10	11	NC
<i>Shigella flexneri</i>	1462	0.05	9	94	374	374	20-fold decrease
<i>Candida albicans</i>	5314	12.5	1	2	4	8	NC
<i>Trichomonas foetus</i>	9644	1.2	1	1			NC

^a Minimal inhibitory concentration determined in STS Medium (BBL) for *T. foetus* (inoculum of 10⁸ cells/ml) and in antibiotic assay broth (BBL) for all other organisms (inoculum of 10⁸ cells/ml).

^b Index of induced resistance = (MIC of final passage)/(MIC of initial passage).

^c Mean lethal dose.

^d No significant change from that of original culture.

less (Table 2). These included 9 (100%) species of gram-positive bacteria, 15 of 18 (83%) gram-negative bacteria, 11 of 19 (58%) fungi, and both strains of *Trichomonas*. All 52 organisms tested, including four for which pathogenicity has not been demonstrated, were susceptible to SQ 18,506 at an MIC of less than 50 µg/ml. Where comparison was possible, SQ 18,506 showed activity in vitro equal to or greater than that of furazolidone.

The data (Table 2) indicate that SQ 18,506 should be considered as a microbicidal agent. There was no significant difference between MIC and MMC in the six examples cited.

A comparison in vitro of the susceptibility to SQ 18,506 of 114 strains of microorganisms known to cause infections of the vagina in humans and animals (Table 3) showed that members of these seven groups of organisms were all susceptible to the drug at less than 1.0 µg/ml (49%), less than 25 µg/ml (68%), or less than 50 µg/ml (100%).

Development of resistance. Attempts to induce resistance produced no significant change in MIC or pathogenicity for mice with *Staphylococcus aureus*, *Salmonella schottmuelleri*, *Candida albicans*, or *Trichomonas foetus* (Table 4). The resistance of *Shigella flexneri* SC 1462 to SQ 18,506 developed rapidly, whereas the resistance of *Escherichia coli* SC 8517 developed gradually, in a stepwise manner. Even though these two strains showed increased resistance to SQ 18,506 after 20 passages in vitro, the pathogenicity of *E. coli* SC 8517 remained the same as that of the original culture, but that of *S. flexneri* was reduced markedly.

Cross-resistance. SQ 18,506 was not cross-resistant with benzylpenicillin, ampicillin, cephalothin, bacitracin, erythromycin, tetracycline, gentamicin, neomycin, kanamycin, chloramphenicol, nitrofurantoin, or sulfamethoxazole, as shown by its effectiveness against organisms resistant to these antimicrobial agents (Table 2 and 5). Some cross-resistance to furazolidone was shown.

TABLE 5. Activity of SQ 18,506 against bacterial variants resistant to various antimicrobial agents

Organism	Resistant to	MIC ^a (µg/ml)	
		SQ 18,506	Furazolidone
<i>Staphylococcus aureus</i> CL-20010	Benzylpenicillin Bacitracin Chloramphenicol Tetracycline Erythromycin	3.2	15.2
<i>Providencia</i> sp. CL-21091	Gentamicin Kanamycin Neomycin Ampicillin Tetracycline	25.0	75.0
<i>Escherichia coli</i> CL-20116	Ampicillin Cephalothin Nitrofurantoin Furazolidone Sulfamethoxazole	1.4	>100
<i>Proteus mirabilis</i> CL-21171	SQ 18,506 Furazolidone Gentamicin Chloramphenicol Tetracycline Kanamycin Cephalothin	>100	>100

^a Minimal inhibitory concentration.

cline, gentamicin, neomycin, kanamycin, chloramphenicol, nitrofurantoin, or sulfamethoxazole, as shown by its effectiveness against organisms resistant to these antimicrobial agents (Table 2 and 5). Some cross-resistance to furazolidone was shown.

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