ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1980, p. 311-316
0066-4804/80/08-0311/06$02.00/0

Vol. 18, No. 2

In Vitro Activity of Chloramphenicol and Thiamphenicol Analogs

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The in vitro activity of three fluorine analogs of chloramphenicol in which the hydroxyl group at position 3 had been replaced with a fluorine was compared with that of chloramphenicol and thiamphenicol. Compound SCH 24893 was the most active agent against staphylococci and *Bacteroides* strains, and compound SCH 25298 was the most active against *Haemophilus*, *Neisseria*, enterococcus, and *Klebsiella* strains. *Serratia marcescens* and *Pseudomonas aeruginosa* strains resistant to chloramphenicol were resistant to the compounds. The agents inhibited all of the *Shigella*, *Salmonella*, *Staphylococcus aureus*, and enterococcus strains resistant to chloramphenicol. They inhibited most (82%) of *Escherichia coli* and half of the *Klebsiella pneumoniae* strains which were resistant to chloramphenicol. Isolates in which resistance to chloramphenicol was shown to be plasmid mediated and due to chloramphenicol transacetylase were inhibited by all three agents.

Although chloramphenicol has proved to be an extremely useful antibiotic for the therapy of many different types of infection, its usefulness in recent years has been reduced markedly by the increasing resistance of the members of the *Enterobacteriaceae*. By 1967, 55% of *Klebsiella*, 47% of *Enterobacter*, and 28% of *Proteus* species at the Boston City Hospital were chloramphenicol resistant (4). Chloramphenicol-resistant *Shigella* strains were first reported in the 1950s, and in the 1970s, *Salmonella typhi* strains resistant to chloramphenicol were reported from many countries (2). Thiamphenicol, an analog of chloramphenicol, has been shown to have in vitro activity quite similar to that of chloramphenicol (5).

The chemical preparation of novel structural variants of the basic D-(*threo*)-2-acylamido-1-aryl-1,3-propanediol by replacement of the hydroxyl group at position 3 with a fluorine atom (Fig. 1) made it possible to determine whether such compounds would be active against organisms susceptible to chloramphenicol and to those in which there is plasmid-mediated resistance (5). This study is an evaluation of several derivatives of chloramphenicol and thiamphenicol.

MATERIALS AND METHODS

SCH 24893, SCH 25298, and SCH 25393 (Fig. 1) were gifts of Schering Corp., as was thiamphenicol. Chloramphenicol was a gift from Parke, Davis & Co. The bacteria were clinical isolates from patients hospitalized at The Columbia-Presbyterian Medical Center. Some *Salmonella* organisms were from previous studies from patients at other hospitals in the northwestern United States.

Minimal inhibitory concentrations (MICs) were determined by use of the agar dilution method. A replicating device applied 10^5 colony-forming units as a spot on Mueller-Hinton agar which contained the antibiotics in twofold-dilution steps. Plates were incubated for 24 h at 35°C. The MIC was the concentration of drug at which there was no growth or growth of fewer than five colonies. Plates containing *Proteus mirabilis* contained 0.2 mM *p*-nitrophenylglycerine. *Bacteroides* cultures were tested by using Mueller-Hinton agar supplemented with 5% sheep blood and 0.5 μg of vitamin K per ml, and they were incubated for 48 h in an anaerobic jar. *Haemophilus* and *Neisseria* strains were tested on Levinthal agar, incubated for 24 h at 35°C.

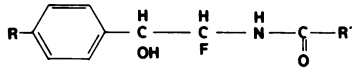
Synergy was determined by using combinations of antibiotics at concentrations similar to those which could be achieved in vivo. Killing curves were performed as previously described in detail (6).

The presence of plasmids mediating resistance in *Enterobacteriaceae* was determined by mating experiments with *Escherichia coli* K-12 strain W1485 by the methods of Anderson (1). Not all isolates were tested, but several strains of *E. coli*, *Shigella*, *Klebsiella*, *Salmonella*, and *Providencia* resistant to 25 μg of chloramphenicol per ml were tested for transferable chloramphenicol resistance. *Staphylococcus aureus* isolates were not tested for the presence of plasmids. The presence of chloramphenicol transacetylase was assayed for by the method of Shaw (7) in a strain of each species which transferred resistance to chloramphenicol.

RESULTS

The comparative activity of the three fluorine derivatives and chloramphenicol and thiam-

phenicol against various gram-negative enteric organisms is shown in Table 1. The range of MICs against *E. coli* was similar for all of the agents. SCH 25393, among the three derivatives, inhibited 75% at 12.5 μg/ml, a concentration of chloramphenicol readily achieved in humans. However, all three fluorine derivatives inhibited 90% of *E. coli* organisms at 25 μg/ml, whereas more than 200 μg of chloramphenicol and thiamphenicol per ml was required to inhibit 90% of isolates. Both chloramphenicol and thiamphenicol inhibited *Klebsiella* isolates at lower concentrations than did the three derivatives. However, 20% of the *Klebsiella* isolates with chloramphenicol MICs above 25 μg/ml were inhibited at that level by all three drugs. The fluorine derivatives against *Enterobacter* were similar to chloramphenicol but more active than thiamphenicol. SCH 25393 was the most active agent of the five against *Citrobacter*, inhibiting 90% of strains at 12.5 μg/ml compared with 100 μg of chloramphenicol per ml. All three derivatives were two- to fourfold more active than chloramphenicol against *P. mirabilis*. Both SCH 25393 and SCH 25298 showed excellent activity against indole-positive *Proteus*, *Proteus morganii*, *Proteus rettgeri*, and *Proteus vulgaris*, with 96 and 92% of isolates inhibited at 12.5 μg/ml compared with only 59% by chloramphenicol and 22% by thiamphenicol at this level. The agents did not inhibit *Serratia marcescens* organisms resistant to chloramphenicol and thiamphenicol. All three derivatives inhibited *Shigella sonnei* organisms (12%) resistant to chloramphenicol, with all isolates inhibited at 12.5 μg/ml. Both SCH 25393 and SCH 25298 inhibited 90% of *Salmonella* strains, including several *S. typhi* strains at 6.3 μg/ml. All of the 22% of chloramphenicol-resistant *Salmonella* strains were inhibited by 6.3 μg of SCH 25393 and SCH 25298 per ml. The majority of the *Providencia* strains tested were chloramphenicol and thiamphenicol resistant (MICs, >25 μg/ml), but SCH 25393 inhibited 82% at 12.5 μg/ml, and SCH 25298 inhibited 68% at this concentration. The fluorine derivatives were not inhibitory against any chloramphenicol- or thiamphenicol-resistant strains of *Pseudomonas aeruginosa*. SCH 24298 inhibited 92% of *Acinetobacter* strains at 12.5 μg/ml, whereas the other two derivatives inhibited only 31% and chloramphenicol inhibited 23% at this concentration. *Bacteroides fragilis* organisms, which included *B. fragilis* subsp. *fragilis*, *B. fragilis* subsp. *thetaiotamicron*, and *B. fragilis* subsp. *distasonis*, were all inhibited by 3.1 μg of the fluorine derivatives per ml, whereas 25 μg of chloramphenicol and thiamphenicol per ml was required to inhibit 90% of isolates.



	R	R
SCH 24893	NO_2	$CHCl_2$
SCH 25298	SO_2CH_3	$CHCl_2$
SCH 25393	SO_2CH_3	CHF_2

FIG. 1. *Structure of chloramphenicol derivatives.*

Table 2 shows the activity of the drugs against other organisms. All three derivatives were more active than chloramphenicol or thiamphenicol, with 90% of isolates inhibited by 3.1 or 6.3 μg of the fluorine derivatives per ml. All of the derivatives inhibited the 20% of isolates which were

TABLE 1. *Comparative activity of chloramphenicol, thiamphenicol, and analog against enteric organisms*

Organism[a]	Antibiotic	Range (μg/ml)	50% MIC (μg/ml)	90% MIC (μg/ml)	% Inhibited at 12.5 μg/ml
E. coli (51)	Chloramphenicol	3.1->200	6.3	>200	74
	Thiamphenicol	1.6->200	100	>200	2
	SCH 24893	6.3-100	12.5	25	55
	SCH 25393	6.3->200	12.5	25	75
	SCH 25298	3.1->200	12.5	25	66
Klebsiella (35)	Chloramphenicol	0.4->200	3.1	>200	61
	Thiamphenicol	6.3->200	3.1	>200	6
	SCH 24893	1.6->200	12.5	50	71
	SCH 25393	3.1->200	12.5	50	77
	SCH 25298	0.8->200	6.3	100	83
Enterobacter (10)	Chloramphenicol	3.1->200	6.3	200	80
	Thiamphenicol	50->200	100	200	0
	SCH 24893	3.1->200	12.5	200	70
	SCH 25393	6.3->200	12.5	200	70
	SCH 25298	3.1->200	12.5	200	80

TABLE 1—*Continued*

Organism[a]	Antibiotic	Range (μg/ml)	50% MIC (μg/ml)	90% MIC (μg/ml)	% Inhibited at 12.5 μg/ml
Citrobacter (16)	Chloramphenicol	3.1->200	6.3	100	75
	Thiamphenicol	12.5->200	100	>200	6
	SCH 24893	3.1-50	25	50	44
	SCH 25393	6.3->200	6.3	12.5	81
	SCH 25298	6.3-100	12.5	25	75
Proteus mirabilis (13)	Chloramphenicol	12.5-25	25	25	38
	Thiamphenicol	12.5->200	200	>200	0
	SCH 24893	12.5-25	12.5	25	46
	SCH 25393	3.1-12.5	6.3	12.5	100
	SCH 25298	6.3-12.5	6.3	12.5	100
Proteus, indole positive (32)	Chloramphenicol	6.3->200	12.5	100	59
	Thiamphenicol	6.3->200	50	>200	22
	SCH 24893	6.3-100	12.5	25	66
	SCH 25393	3.1-25	3.1	6.3	96
	SCH 25298	3.1-25	3.1	12.5	92
Serratia (16)	Chloramphenicol	>200			0
	Thiamphenicol	>200			0
	SCH 24893	>200			0
	SCH 25393	>200			0
	SCH 25298	>200			0
Shigella (32)	Chloramphenicol	0.8->200	3.1	>200	88
	Thiamphenicol	0.8->200	3.1	>200	78
	SCH 24893	1.6-12.5	12.5	12.5	100
	SCH 25393	0.8-6.3	3.1	6.3	100
	SCH 25298	0.8-6.3	6.3	6.3	100
Salmonella (32)	Chloramphenicol	3.1->200	6.3	100	78
	Thiamphenicol	25->200	50	>200	0
	SCH 24893	12.5-25	25	25	41
	SCH 25393	3.1-6.3	6.3	6.3	100
	SCH 25298	3.1-12.5	6.3	6.3	100
Providencia (28)	Chloramphenicol	12.5->200	50	>200	4
	Thiamphenicol	6.3->200	>200	>200	28
	SCH 24893	100->200	>200	>200	0
	SCH 25393	3.1-100	6.3	25	82
	SCH 25298	3.1-100	12.5	50	68
Acinetobacter (13)	Chloramphenicol	3.1->200	50	>200	23
	Thiamphenicol	100->200	100	>200	0
	SCH 24893	0.8-50	12.5	12.5	92
	SCH 25393	3.1-200	>200	>200	31
	SCH 25298	3.1->200	>200	>200	31
P. aeruginosa (10)	Chloramphenicol	12.5->200	>200	>200	
	Thiamphenicol	50->200	>200	>200	
	SCH 24893	12.5->200	>200	>200	
	SCH 25393	25->200	>200	>200	
	SCH 25298	12.5->200	>200	>200	
Bacteroides (51)	Chloramphenicol	3.1-50	6.3	25	87
	Thiamphenicol	1.6-50	6.3	25	71
	SCH 24893	<0.4-3.1	1.6	3.1	100
	SCH 25393	3.1-12.5	6.3	12.5	100
	SCH 25298	0.8-6.3	3.1	3.1	100

[a] The number of isolates is given within parentheses.

TABLE 2. *Activity of chloramphenicol and analogs against various bacteria*

Organism[a]	Antibiotic	Range (μg/ml)	50% MIC (μg/ml)	90% MIC (μg/ml)	% Inhibited at 12.5 μg/ml
S. aureus (15)	Chloramphenicol	0.8–50	6.3	50	80
	Thiamphenicol	1.6–>200	25	>200	50
	SCH 24893	<0.4–3.1	1.6	3.1	100
	SCH 25393	0.8–6.3	3.1	6.3	100
	SCH 25298	0.4–6.3	3.1	6.3	100
Enterococci (15)	Chloramphenicol	6.3–50	6	50	80
	Thiamphenicol	6.3–>100	6.3	>200	0
	SCH 24893	6.3–12.5	1.6	3.1	80
	SCH 25393	3.1–12.5	6.3	12.5	100
	SCH 25298	3.1	3.1	3.1	100
H. influenzae (15)	Chloramphenicol	<0.4–1.6	1.6	1.6	100
	Thiamphenicol	0.8–25	1.6	25	0
	SCH 24893	0.8	0.8	0.8	100
	SCH 25393	3.1–63	3.1	3.1	100
	SCH 25298	<0.4–0.8	0.8	0.8	100
Neisseria gonorrhoeae (15)	Chloramphenicol	1.6–25	6.3	6.3	100
	Thiamphenicol	25–50	6.3	12.5	100
	SCH 24893	0.8–3.1	0.8	3.1	100
	SCH 25393	3.1–25	3.1	6.3	100
	SCH 25298	0.8–3.1	1.6	3.1	100

[a] The number of isolates is given within parentheses.

TABLE 3. *Comparative activity against resistant bacteria*

Organism	MIC (μg/ml)				
	Chloramphenicol	Thiamphenicol	SCH 24893	SCH 25298	SCH 25393
Escherichia coli	100	>200	6.3	3.1	3.1
Enterobacter cloacae	>200	>200	12.5	12.5	12.5
Klebsiella pneumoniae	>200	>200	6.3	3.1	6.3
Providencia	>200	>200	12.5	12.5	6.3
Serratia marcescens	>200	>200	>200	>200	>200
Pseudomonas aeruginosa	100	200	200	100	100
Salmonella typhimurium	>200	>200	6.3	3.1	3.1
Proteus rettgeri	>200	>200	12.5	12.5	12.5
P. vulgaris	>200	>200	12.5	12.5	12.5
Acinetobacter	100	>200	6.3	100	100
Shigella sonnei	100	>200	6.3	3.1	6.3
Citrobacter freundii	>200	>200	25	12.5	12.5
Staphylococcus aureus	50	200	3.1	3.1	6.3
Bacteroides melaninogenicus	50	50	3.1	12.5	6.3
Salmonella typhi	>100	>100	6.3	3.1	3.1

chloramphenicol resistant (MICs, ≥ 25 μg/ml). SCH 25393 and SCH 25298 inhibited all enterococci at a concentration of 12.5 μg/ml. Only 80% were inhibited by chloramphenicol at this level. All of the *Haemophilus influenzae* organisms were susceptible to chloramphenicol. However, SCH 24893 and SCH 25298 were twofold more active than chloramphenicol and eightfold more active than SCH 25393. All three derivatives were more active against *Neisseria* strains than was chloramphenicol or thiamphenicol, but all compounds inhibited all isolates at 12.5 μg/ml.

The comparative activity of the compounds against chloramphenicol-resistant isolates is shown in Table 3. Except for *P. aeruginosa*, *S. marcescens*, and *Acinetobacter*, the chloramphenicol-resistant bacteria were inhibited at concentrations which should be able to be achieved in animals in view of the blood levels in animals (G. Miller, personal communication). Table 4 shows the activity of the derivatives

against isolates with chloramphenicol MICs above 25 μg/ml. The derivatives inhibited most of the *E. coli*, half of the *Klebsiella*, and all of the *Shigella*, *Salmonella*, and *S. aureus* strains resistant to chloramphenicol at this level.

When the three compounds were tested in combination with gentamicin against various organisms (Table 5), there was no increase in the gentamicin MIC for any of 11 organisms. In only one case for each compound was there an increase in the MIC of the chloramphenicol derivative in the presence of the aminoglycoside.

TABLE 4. *Activity of chloramphenicol derivatives resistant to 25 μg of chloramphenicol per ml*

Organism[a]	% Resistant		
	SCH 24893	SCH 25393	SCH 25298
E. coli (11)	27	18	18
Shigella (4)	0	0	0
Klebsiella (13)	54	46	46
Enterobacter (2)	100	100	100
Salmonella (7)	0	0	0
Citrobacter (2)	100	50	50
Providencia (20)	0	15	25
Pseudomonas (7)	6	100	100
Acinetobacter (8)	11	100	100
S. aureus (3)	0	0	0
Enterococcus (1)	0	0	0
Bacteroides (2)	0	0	0

[a] The number of isolates is given within parentheses.

DISCUSSION

Chloramphenicol has proved to be an extremely useful antibiotic because of its broad spectrum of activity against gram-positive and -negative aerobic and anaerobic species. However, the presence in many enteric bacteria of a plasmid-mediated inactivating enzyme, chloramphenicol transacetylase, has decreased the usefulness of the compound (8). This study demonstrates that chloramphenicol derivatives in which the hydroxyl group at position 3 of the basic structure has been replaced with a fluorine atom are active gainst certain organisms resistant to chloramphenicol and thiamphenicol. Of the derivatives tested here, SCH 24893 was the most active against staphylococci and *Bacteroides* strains, and SCH 25298 was the most active against *Haemophilus*, *Neisseria*, enterococcus, and *Klebsiella* strains. *S. marcescens* strains which were resistant to compounds of most antibiotic classes were resistant. The differences in activity of thiamphenicol and chloramphenicol which we found are in contrast to those of other workers (3, 5) and are not explained.

The precise role for such compounds is not clear in view of the development of relatively nontoxic broad-spectrum cephalosporins which will inhibit greater numbers of bacteria. However, it is possible that agents such as these could be useful in veterinary medicine, in which the ease of administration and wide tissue distribution of such compounds would be beneficial in curing animal infections. Whether such agents might play a role in central nervous system infections would depend upon their pharmacokinetic properties.

TABLE 5. *Effect of SCH 24893, SCH 25298, and SCH 25393 combined with gentamicin*

Organism	MIC (μg/ml)[a]						
	Gentamicin	SCH 24893	SCH 24893 plus gentamicin	SCH 25298	SCH 25298 plus gentamicin	SCH 25393	SCH 25393 plus gentamicin
Shigella 11-32	0.8	12.5	3.1/0.8	6.3	3.1/0.8	3.1	3.1/0.8
Shigella 8-121	1.6	12.5	3.1/0.8	6.3	3.1/0.8	3.1	3.1/0.8
Salmonella 3697	0.4	25	1.6/0.4	12.5	1.6/0.4	6.3	1.6/0.4
Salmonella 2889	0.4	12.5	1.6/0.4	6.3	1.6/0.4	3.1	3.1/0.8
Enterobacter 3964	0.8	>200	1.6/0.4	200	1.6/0.4	100	1.6/0.4
Pseudomonas 3904	1.6	>200	3.1/0.8	200	3.1/0.8	>200	3.1/0.8
Staphylococcus epidermidis 4005	12.5	3.1	3.1/0.8	6.3	3.1/0.8	6.3	12.5/3.1
Klebsiella 4030	25	12.5	12.5/3.1	3.1	3.1/0.8	12.5	6.3/1.6
Klebsiella 3528	>25	50	12.5/3.1	6.3	6.3/1.6	12.5	6.3/1.6
E. coli 3494	25	25	12.5/3.1	200	12.5/3.1	>200	12.5/3.1
E. coli 3911	0.8	50	1.6/0.4	25	1.6/0.4	25	1.6/0.4

[a] MICs were determined by the agar dilution method in Mueller-Hinton agar.

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