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In Vitro Activities of Two New Glycylcyclines, *N,N*-Dimethylglycylamido Derivatives of Minocycline and 6-Demethyl-6-Deoxytetracycline, against 339 Strains of Anaerobic Bacteria

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The in vitro activities of the *N,N*-dimethylglycylamido derivatives of minocycline (DMG-MINO) and 6-demethyl-6-deoxytetracycline (DMG-DMDOT) were compared with those of minocycline, tetracycline, clindamycin, and metronidazole by using the National Committee for Clinical Laboratory Standards-approved Wadsworth agar dilution method. The MICs of DMG-MINO, DMG-DMDOT, and metronidazole at which 90% of the strains were susceptible (0.5, 1, and 1 μg/ml, respectively) were lower than those for clindamycin, minocycline, and tetracycline (4, 8, and 32 μg/ml, respectively). All of the strains of anaerobes tested, except one strain of *Bacteroides ovatus* (MIC, 16 μg/ml), were susceptible to DMG-MINO and DMG-DMDOT at 8 μg/ml.

Two new antimicrobial agents, *N,N*-dimethylglycylamido derivatives of minocycline (DMG-MINO) and 6-demethyl-6-deoxytetracycline (DMG-DMDOT), have been synthesized by American Cyanamid, Inc. (5). Data from the manufacturer indicate that these compounds are active against gram-positive and gram-negative aerobic and anaerobic bacteria, including those organisms carrying the most common tetracycline resistance determinants (3, 5). The purpose of this study was to evaluate the in vitro activities of these compounds against a large group of fresh clinical isolates of anaerobic bacteria.

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Antimicrobial agents were obtained as powders from the following sources: DMG-MINO, DMG-DMDOT, and minocycline were from American Cyanamid Inc., Pearl River, N.Y.; clindamycin was from Upjohn, Kalamazoo, Mich.; and metronidazole and tetracycline were from Sigma, St. Louis, Mo. All bacteria were randomly selected recent clinical isolates from the Veterans Administration Wadsworth Medical Center, Los Angeles, Calif. Bacteria were identified in accordance with established procedures (1, 4). MICs were determined by an agar dilution technique described previously by using an inoculum of 10^5 CFU (as specified in the National Committee for Clinical Laboratory Standards guidelines [2]) and brucella base-laked blood agar. *Bacteroides gracilis* strains were tested on brucella base-laked blood agar with fumarate and formate (each at 0.3%) added; *Bilophila wadsworthia* was tested on brucella base-laked blood agar with pyruvate (1%). Plates were incubated in an anaerobic chamber (Anaerobe Systems, San Jose, Calif.) for 48 h at 37°C. The MIC was defined, in accordance with National Committee for Clinical Laboratory Standards guidelines, as the lowest antimicrobial agent concentration producing a marked change from the growth control (2). Reference strains of *Bacteroides fragilis* (ATCC 25285) and *B. thetaiotaomicron* (ATCC 29741) were used as controls in each test.

The in vitro activities of the agents tested are listed in Table 1. No breakpoints have been established for the new glycylcycline compounds; the National Committee for Clinical Laboratory Standards-approved breakpoints for clindamycin, tetracycline, and metronidazole are 4, 8, and 16 μg/ml, respectively. DMG-MINO was more active than minocycline. The MIC of DMG-MINO at which 90% of the strains were susceptible (MIC_{90}) was as much as 7 twofold dilutions lower than the MIC_{90} of minocycline (for *Porphyromonas* species, 0.062 and 8 μg/ml, respectively). The MIC_{90} of DMG-MINO for all of the anaerobes tested was 0.5 μg/ml, compared with 8 μg/ml for minocycline.

DMG-DMDOT demonstrated much better activity than tetracycline against most groups of organisms. For all of the strains tested, the MIC_{90}s of DMG-DMDOT and tetracycline were 1.0 and 32 μg/ml, respectively.

The two new glycylcycline compounds demonstrated excellent activity against anaerobes. DMG-MINO, DMG-DMDOT, metronidazole, and clindamycin inhibited all of the strains of *Prevotella* species tested at 8 μg/ml, compared with 82 and 64% for minocycline and tetracycline, respectively. All 9 strains of *B. gracilis*, 16 strains of *Fusobacterium nucleatum*, 12 strains of *F. mortiferum-F. varium*, and 30 strains of *Bilophila wadsworthia* tested were inhibited by all of the antimicrobial agents at 8 μg/ml, except for tetracycline. Ten *Clostridium difficile* strains were inhibited at 8 μg/ml by DMG-MINO, DMG-DMDOT, and metronidazole but only 50% were inhibited by clindamycin, minocycline, and tetracycline. All of the strains of *B. ovatus* tested but one (MIC,

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TABLE 1. Activities of antimicrobial agents against anaerobic organisms[a]

Organism (no. of strains) and antimicrobial agent	MIC range	MIC_{50}	MIC_{90}
Bacteroides fragilis (49)			
DMG-MINO	0.062–4	0.5	1
DMG-DMDOT	0.062–8	0.5	2
Clindamycin	0.062–>32	0.5	1
Metronidazole	0.25–2	1	1
Minocycline	0.062–16	8	8
Tetracycline	0.25–64	32	64
Other *B. fragilis* group species (74)[b]			
DMG-MINO	0.062–16	0.25	1
DMG-DMDOT	0.062–16	0.5	1
Clindamycin	0.062–>32	2	8
Metronidazole	0.062–2	1	1
Minocycline	0.062–32	4	8
Tetracycline	0.125–>64	16	32
Bacteroides gracilis (9)			
DMG-MINO	0.062–0.062	0.062	
DMG-DMDOT	0.062–0.062	0.062	
Clindamycin	0.062–0.125	0.125	
Metronidazole	0.062–0.5	0.062	
Minocycline	0.062–0.25	0.062	
Tetracycline	0.062–0.5	0.125	
Other *Bacteroides* species (12)[c]			
DMG-MINO	0.062–1	0.062	0.125
DMG-DMDOT	0.062–0.5	0.125	0.25
Clindamycin	0.062–0.5	0.062	0.25
Metronidazole	0.125–4	0.125	1
Minocycline	0.125–16	0.5	4
Tetracycline	0.25–64	0.5	32
Porphyromonas species (11)[d]			
DMG-MINO	0.062–0.062	0.062	0.062
DMG-DMDOT	0.062–0.125	0.062	0.125
Clindamycin	0.062–>32	0.062	0.125
Metronidazole	0.062–0.125	0.125	0.125
Minocycline	0.062–8	0.125	8
Tetracycline	0.125–16	0.5	16
Prevotella species (32)[e]			
DMG-MINO	0.062–1	0.125	0.5
DMG-DMDOT	0.062–4	0.25	2
Clindamycin	0.062–0.062	0.062	0.062
Metronidazole	0.062–8	0.5	2
Minocycline	0.062–16	0.5	16
Tetracycline	0.125–>64	1	64
Fusobacterium nucleatum (16)			
DMG-MINO	0.062–0.125	0.062	0.125
DMG-DMDOT	0.062–0.5	0.125	0.25
Clindamycin	0.062–0.125	0.062	0.125
Metronidazole	0.062–0.125	0.125	0.125
Minocycline	0.062–0.25	0.125	0.25
Tetracycline	0.125–1	0.5	1
Fusobacterium mortiferum-F. varium group (12)			
DMG-MINO	0.062–0.25	0.125	0.25
DMG-DMDOT	0.125–0.5	0.5	0.5
Clindamycin	0.062–4	0.062	4
Metronidazole	0.125–0.5	0.25	0.5
Minocycline	0.062–8	0.125	8
Tetracycline	0.25–16	0.5	16

Continued

TABLE 1—*Continued*

Organism (no. of strains) and antimicrobial agent	MIC range	MIC_{50}	MIC_{90}
Other *Fusobacterium* species (8)[f]			
DMG-MINO	0.062–0.25	0.062	
DMG-DMDOT	0.062–1	0.125	
Clindamycin	0.062–1	0.062	
Metronidazole	0.125–0.25	0.125	
Minocycline	0.125–8	0.125	
Tetracycline	0.125–8	0.5	
Bilophila wadsworthia (30)			
DMG-MINO	0.062–0.25	0.125	0.125
DMG-DMDOT	0.062–0.25	0.125	0.25
Clindamycin	0.062–4	0.25	0.5
Metronidazole	0.062–0.25	0.125	0.125
Minocycline	0.125–4	0.5	1
Tetracycline	0.125–16	0.25	4
Clostridium difficile (10)[g]			
DMG-MINO	0.062–0.5	0.125	0.25
DMG-DMDOT	0.125–1	0.25	1
Clindamycin	2–>32	4	>32
Metronidazole	0.125–1	0.25	0.25
Minocycline	0.125–16	1	16
Tetracycline	0.125–>64	4	64
Clostridium perfringens (10)			
DMG-MINO	0.125–4	0.5	4
DMG-DMDOT	0.125–4	0.5	4
Clindamycin	0.062–4	0.25	2
Metronidazole	0.25–2	0.5	1
Minocycline	0.125–32	0.25	16
Tetracycline	0.125–32	8	32
Clostridium ramosum (10)			
DMG-MINO	0.125–0.25	0.125	0.25
DMG-DMDOT	0.125–0.5	0.25	0.5
Clindamycin	2–>32	4	>32
Metronidazole	0.5–2	0.5	1
Minocycline	0.125–16	0.125	16
Tetracycline	0.5–64	0.5	64
Other *Clostridium* species (5)[h]			
DMG-MINO	0.062–0.062	0.062	
DMG-DMDOT	0.062–0.062	0.062	
Clindamycin	0.062–2	0.5	
Metronidazole	0.125–0.5	0.25	
Minocycline	0.125–2	0.125	
Tetracycline	0.125–32	0.125	
Peptostreptococcus species (25)[i]			
DMG-MINO	0.062–0.25		
DMG-DMDOT	0.062–0.5	0.125	0.5
Clindamycin	0.062–>32	0.125	2
Metronidazole	0.062–2	0.25	1
Minocycline	0.125–16	0.5	8
Tetracycline	0.125–64	4	32
Gram-positive rods (non-spore forming) (23)[j]			
DMG-MINO	0.062–0.5	0.125	0.25
DMG-DMDOT	0.062–1	0.25	0.5
Clindamycin	0.062–2	0.125	1
Metronidazole	0.125–>128	4	>128
Minocycline	0.125–8	0.125	8
Tetracycline	0.25–32	1	32
Total (339)			
DMG-MINO	0.062–16	0.125	0.5
DMG-DMDOT	0.062–16	0.25	1
Clindamycin	0.062–>32	0.25	4
Metronidazole	0.062–>128	0.5	1

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TABLE 1—*Continued*

Organism (no. of strains) and antimicrobial agent	MIC range	MIC_{50}	MIC_{90}
Minocycline	0.062–32	0.5	8
Tetracycline	0.062–>64	4	32

[a] All MICs are in micrograms per milliliter.

[b] Includes 3 *B. caccae*, 11 *B. distasonis*, 1 *B. eggerthii*, 7 *B. ovatus*, 3 *B. stercoris*, 28 *B. thetaiotaomicron*, 8 *B. uniformis*, and 13 *B. vulgatus* strains.

[c] Includes two *B. capillosus*, five *B. splanchnicus*, two *B. ureolyticus*, and two other *Bacteroides* species strains.

[d] Includes four *Porphyromonas asaccharolytica*, two *P. endodontalis*, and four *P. gingivalis* strains and one other *Porphyromonas* species strain.

[e] Includes four *Prevotella bivia*, one *P. buccae*, two *P. corporis*, two *P. denticola*, one *P. disiens*, eight *P. intermedia*, five *P. loeschii*, and five *P. melaninogenica* strains; one *P. oralis*, one *P. oris*, and one *P. zoogleoformans* strain; and one other *Prevotella* species strain.

[f] Includes three *F. necrophorum*, one *F. gonidiaformans*, one *F. necrogenes*, and three other *Fusobacterium* species strains.

[g] *C. difficile* is of interest primarily in relation to antimicrobial agent-induced pseudomembranous colitis. These data must be interpreted in the context of the level of the drug achieved in the colon and the impact of the agent on indigenous colonic flora.

[h] Includes two *C. innocuum* strains and one *C. bifermentans*, one *C. sordellii*, and one other *Clostridium* species strain.

[i] Includes six *Peptostreptococcus micros*, five *P. magnus*, four *P. prevotii*, four *P. asaccharolyticus*, three *P. anaerobius*, and three other *Peptostreptococcus* species strains.

[j] Includes three *Actinomyces israelii*, three *A. odontolyticus*, one other *Actinomyces* species, one *Eubacterium alactolyticum*, three *E. lentum*, two *E. limosum*, one other *Eubacterium* species, two *Lactobacillus catenaforme*, two *L. minutus*, one other *Lactobacillus* species, and four *Propionibacterium acnes* strains.

16 μg/ml) were inhibited at ≤8 μg/ml by both DMG-MINO and DMG-DMDOT. The utility of these compounds for therapy of anaerobic infections merits further investigation.

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