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SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIP OF NOVEL GLYCYLCYCLINE DERIVATIVES LEADING TO THE DISCOVERY OF GAR-936

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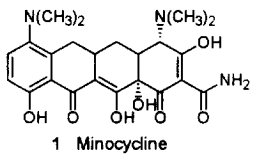
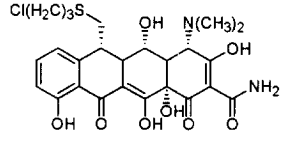
Abstract: A number of new glycylcyclines were synthesized for structure–activity relationship study. Many of the derivatives exhibit potent, broad spectrum antibacterial activity against both tetracycline susceptible and resistant organisms. GAR-936 (TBG-MINO) shows better activity than the previously reported DMG-MINO and DMG-DMDOT.

Tetracyclines are a group of clinically useful, broad spectrum antibiotics which prevent bacterial growth by inhibiting protein biosynthesis. These agents have been important medical products for the last 40 years. In recent years, however, the emergence of resistant bacteria has diminished the utility of most tetracyclines.[1,2] The widely used minocycline **1**, discovered in our laboratories three decades ago, has also become ineffective against certain resistant strains.[2] There are two established mechanisms of bacterial resistance to tetracyclines: (1) efflux of antibiotics by membrane-spanning proteins, and (2) ribosomal protection. Inhibitors of efflux action by tetracycline derivatives such as **2**, had been reported.[3] Our approach to overcome both mechanisms of resistance centers on the development of new tetracycline derivatives through modifications of minocycline. We report herein the discovery of GAR-936 as a novel tetracycline with potent activity against resistant bacterial pathogens.



1 Minocycline



2

Previously, we reported a series of novel tetracycline derivatives which have been referred to as "glycylcyclines". These compounds represent a significant advance within their class of antibiotics due to their activity against tetracycline resistant organisms with either the ribosomal protection or the efflux mechanism of resistance.[4,5] Two such derivatives DMG-MINO (9-*N,N*-dimethylglycylamido-minocycline) **3** and DMG-DMDOT (9-*N,N*-dimethylglycylamido-6-demethyl-6-deoxytetracycline) **4** have been studied by numerous investigators, and have shown improved activity over minocycline when tested against a wide spectrum of gram-positive and gram-negative aerobic and anaerobic clinical bacterial isolates.[6]

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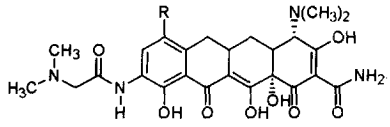
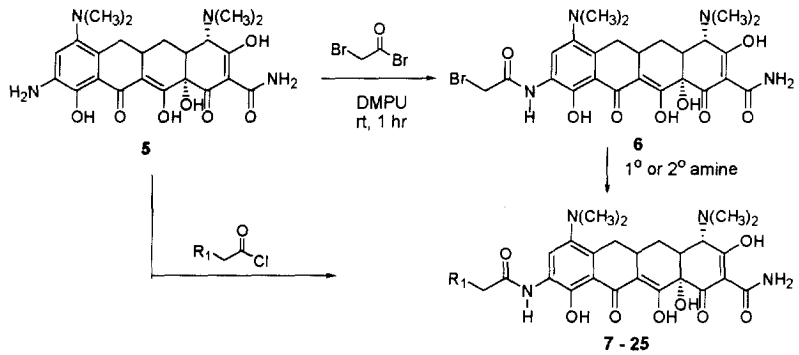


3 R = H, DMG-DMDOT
4 R = $N(CH_3)_2$, DMG-MINO

In continuation of our program to search for clinically useful tetracyclines and to extend the structure–activity relationship studies of glycylcyclines, a number of compounds with modifications of the 9-glycyl moiety were synthesized. In this communication, the synthesis and activity of selected derivatives prepared from minocycline will be discussed, tetracycline and minocycline **1** are included for comparison. These compounds were synthesized via the following synthetic routes. Reaction of 9-amino-minocycline **5**[4,7] with 2.5 equivalents of bromoacetyl bromide in DMPU (*N,N*-dimethylpropyleneurea) gave the bromo compound **6** in greater than 90 % yield. Compounds **7–25** were prepared by treating **6** with the appropriate primary or secondary amines in moderate to good yields. A commercially viable purification method was developed for the isolation of the final products. This was achieved by gradient pH extraction, and the desired products isolated from fractions between pH 4.5–6.5 were greater than 90% pure. In an alternative synthetic route, *t*-butylaminoacetyl chloride was reacted directly with **5** to give TBG-MINO **16** in yields comparable to the previous method. This less costly route combined with the efficient purification method, has been applied to the scale-up synthesis of **16** (TBG-MINO, also referred to as GAR-936).



5 **6** **7 - 25**

Table 1 shows the in vitro antibacterial activity of compounds synthesized from the reaction of bromo compound **6** with a number of secondary amines. Compounds **7** and **9** exhibit potent broad spectrum activity comparable to that of DMG-MINO **3**. The pyrrolidinyl derivative **8** shows better activity than DMG-MINO, while the imidazolyl **10**, morpholinyl **11** or thiomorpholinyl **12** derivatives are only slightly active against Gram-(+) bacteria and are almost inactive against Gram-(-) bacteria. The intermediate compound **6** shows good activity against *Staphylococcus aureus* and *Enterococcus faecalis*, yet is not active against *Escherichia coli*, indicating that the amino function is crucial to the activity against Gram-(-) organisms.

Table 1. In Vitro Antibacterial Activity of Compounds **6–12**

		Organism; minimum inhibitory concentration (MIC) μg/mg										
	R_1	***E. coli*** **UBMS 88-1 Tet B**	***E. coli*** **PRP1 Tet A**	***E. coli*** **J3272 Tet C**	***E. coli*** **J3272 Tet D**	***E. coli*** **UBMS 90-4 Tet M**	***E. coli*** **UBMS 90-5 sensitive**	***S. aureus*** **UBMS 88-7 Tet K**	***S. aureus*** **UBMS 90-1 Tet M**	***S. aureus*** **UBMS 90-3 sensitive**	***S. aureus*** **Smith sensitive**	**Entero-coccus ATCC 29212**
6	Br	>32	>32	>32	32	NT	16	2	1	0.5	0.5	0.5
4	Me_2N	0.25	1	1	0.12	0.25	0.25	0.25	0.25	0.03	0.06	0.06
7		0.5	1	0.5	0.12	0.25	0.25	2	0.25	0.12	0.25	0.25
8		0.25	0.5	0.25	0.12	0.25	0.25	0.5	0.12	0.12	0.12	0.12
9		0.5	1	0.25	0.5	0.5	0.5	0.25	0.25	0.12	0.12	0.06
10		>32	>32	>32	32	>32	>32	32	32	4	4	4
11		>32	>32	>32	32	NT	>32	32	4	2	2	2
12		>32	>32	>32	16	>32	>32	4	1	1	0.5	0.25
	tetracycline	>32	32	>32	8	>32	1	>32	>32	0.25	0.25	16
1	minocycline	>32	>32	32	4	16	16	16	8	2	2	4

Compounds **13–25**, synthesized from the reaction of primary amines with bromo compound **6**, are listed in Table **2**. In this series, the methylamino derivative **13** is considerably less active than the dimethylamino derivative (DMG-MINO) **4**, while the more lipophilic compounds **14, 15** and **17** show activities comparable to that of DMG-MINO. The longer chain *n*-undecylamino compound **18** is much less active. In the case of compounds with cycloalkyl or heterocyclic substitutions, those with compact rigid ring structures (**20** and **22**) show the best activity. The pyridinyl compound **25** is only moderately active, but is slightly more active than the imidazolyl derivative **10**.

In general, the attachment of an *N*-alkyl glycylamido group to the 9-position of the tetracycline nucleus produced compounds with excellent antibacterial activity against a broad spectrum of tetracycline susceptible and resistant Gram-(-) and Gram-(+) bacteria, including strains of *E. coli* and *S. aureus* containing *tet*M (ribosomal) resistance determinants, in *E. coli* containing *tet*A-D, and in *S. aureus* with *tet*K (efflux) resistance determinants. The most active compound of this series is the 9-*t*-butylglycylamido derivative (TBG-MINO or GAR-936) **16**. Extensive in vitro and in vivo testing has been conducted with GAR-936, which has demonstrated efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant streptococci and vancomycin-resistant enterococci and GAR-936 is currently in clinical trials. The results of these experiments are being communicated separately.[8]

Table 2. In Vitro Antibacterial Activity of Compounds **13–25**.

		Organism; minimum inhibitory concentration (MIC) μg/mg										
	R_1	***E. coli*** **UBMS 88-1 Tet B**	***E. coli*** **PRP1 Tet A**	***E. coli*** **J3272 Tet C**	***E. coli*** **J3272 Tet D**	***E. coli*** **UBMS 90-4 Tet M**	***E. coli*** **UBMS 90-5 sensitive**	***S. aureus*** **UBMS 88-7 Tet K**	***S. aureus*** **UBMS 90-1 Tet M**	***S. aureus*** **UBMS 90-3 sensitive**	***S. aureus*** **Smith sensitive**	**Entero-coccus ATCC 29212**
13	MeNH	1	16	8	0.5	NT	1	16	1	0.5	0.5	0.5
14	*n*-PrNH	0.5	2	0.5	0.12	0.25	0.5	2	0.5	0.25	0.25	0.25
15	*n*-BuNH	0.5	1	0.5	0.25	0.25	0.5	2	0.5	0.25	0.12	0.12
16	*t*-BuNH	0.5	0.25	0.25	0.12	0.12	0.25	0.5	0.12	0.25	0.25	0.12
17	*n*-HexylNH	0.5	0.5	0.5	0.12	0.25	0.25	2	0.25	0.06	0.12	0.12
18	UndecylNH	32	32	32	32	32	16	2	16	0.5	0.5	2
19		4	32	8	2	2	2	4	0.5	0.5	0.25	0.25
20		0.25	1	0.25	0.12	0.25	0.25	2	0.25	0.12	0.25	0.12
21		4	2	2	0.5	2	4	0.5	1	0.25	0.5	0.25
22		0.5	1	0.5	0.25	0.5	0.5	0.5	0.5	0.25	0.25	0.12
23		0.5	4	0.5	0.25	0.5	0.5	4	1	0.5	0.5	0.25
24	$PhCH_2NH$	2	4	2	0.5	0.5	0.5	2	0.5	0.25	0.25	0.25
25		16	32	16	8	8	16	32	8	4	4	2

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