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Bioorganic & Medicinal Chemistry Letters 16 (2006) 400–403
Bioorganic & Medicinal Chemistry Letters

Synthesis and antibacterial activity of 9-substituted minocycline derivatives

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Received 15 August 2005; revised 20 September 2005; accepted 26 September 2005
Available online 21 October 2005

Abstract—A number of 9-acylamino and 9-sulfonylamino derivatives of minocycline have been synthesized for structure–activity relationship studies. These compounds showed activity against both tetracycline-susceptible and tetracycline-resistant strains. Many of the 9-sulfonylamino derivatives exhibited improved antibacterial activity against a number of tetracycline- and minocycline-resistant Gram-positive pathogens.

The first tetracycline was introduced in the 1950s as a safe and effective broad-spectrum antibiotic, and it has been more than 30 years since the last tetracycline antibiotic, minocycline (**1**), was introduced into the market.[1] These agents have been important medical products for the last 50 years, however, the utility of these agents has gradually diminished in recent years due to the emergence of tetracycline-resistant bacteria. Similarly, the alarming incidence of pathogenic bacteria resistant to other currently available antibiotics has caused concern among medical professionals.[2] Tetracyclines inhibit protein synthesis by interfering with the binding of aminoacylated tRNA to the A-site of the 30S subunit. There are two major resistance mechanisms associated with tetracyclines: (1) ribosome protection mechanism mediated by cytoplasmic protein that interacts with the ribosome, and (2) efflux of the antibiotics mediated by membrane-spanning proteins (an active transport mechanism).[3–6]

Previously, we reported a novel class of semi-synthetic tetracyclines that has been referred to as 'glycylcyclines'.[7] The unique feature of these agents was the ability to overcome both ribosomal protection (*tet*M) and efflux resistance (*tet*A, *tet*B, *tet*C, *tet*D, *tet*K, etc.) mechanisms associated with tetracycline. Most importantly, glycylcyclines are also effective against pathogens that are resistant to other antibiotics, for example, methicillin-resistant *Staphylococci*, penicillin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococci*.[8] Three of the glycylcyclines, DMG-DMDOT **2**, DMG-MINO **3**, and TBG-MINO **4** (also known as tigecycline or GAR-936), have been investigated extensively.[9,10] Tigecycline (Tygacil®) was selected for further development (Fig. 1) and was recently approved by the FDA.

During the course of our research to discover compounds with improved activity and pharmacokinetic properties, we have synthesized a number of compounds with C-9 modifications. These compounds were designed based on previous SAR that modifications at ring-D might lead to compounds with enhanced activity.[11] The crystal structure of tetracycline binding to 30S ribosomal subunit was only recently determined by Ramakrishnan and co-workers.[12] The data obtained from the crystal structure shared numerous similarities with our hypothesis derived from SAR and many biochemical experiments.[13,14] Crystal structure of the *tet* repressor in complex with DMG-DMDOT indicated that the bulky and positively charged glycylamido group interferes with amino acid side chains which were identified by mutagenesis as sensitive to Tc (tetracycline) recognition and to the mechanism of induction.[15] Both findings suggested that the hydrophilic domain of the tetracycline molecule is important for binding to ribosome (Fig. 2) and modification at that region would

Keywords: Novel tetracyclines; Antibiotic; Resistant bacteria.
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doi:10.1016/j.bmcl.2005.09.078

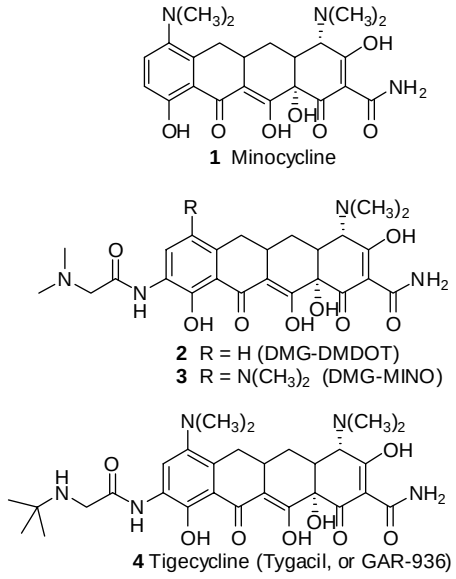


Figure 1. Structures of minocycline (**1**), DMG-DMDOT (**2**), DMG-MINO (**3**), and tigecycline or tygacil® (**4**).

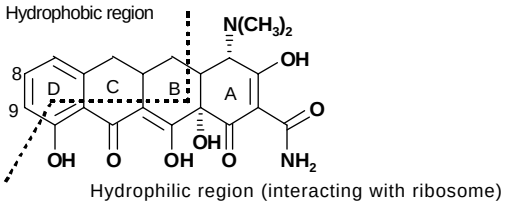


Figure 2. Possible interactions of 6-demethyl-6-deoxytetracycline with 30S ribosomal subunit.

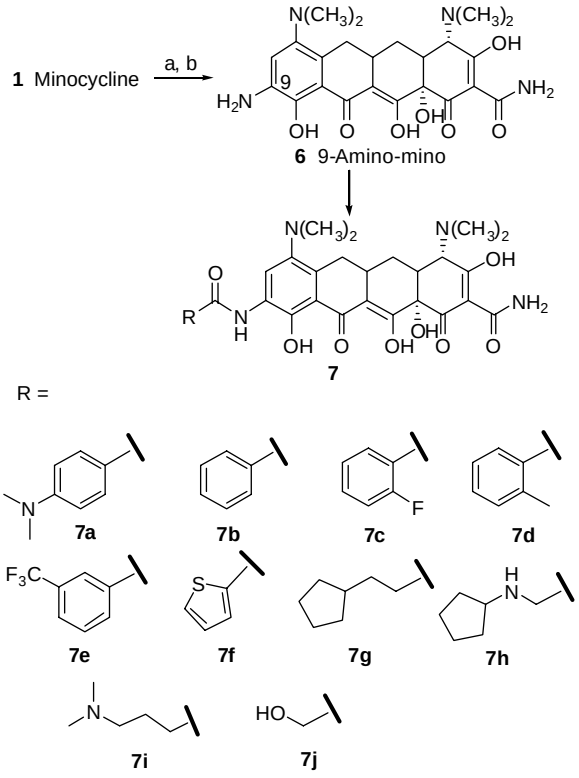
interfere with the binding, while modification of the hydrophobic region might produce compounds with enhanced antibacterial activity.

Herein, we report the synthesis and antibacterial activity of 9-acylamino and 9-sulfonylamino derivatives of minocycline. The key intermediate was prepared using the same method as described previously.[7] Nitration of minocycline **1** gave the 9-nitro-mino and subsequent catalytic reduction gave the desired 9-amino-mino **6**. Treatment of 9-amino-mino hydrochloride **6** with excess sodium carbonate and appropriate acylating agents gave the desired final products **7a**–**g** and **j** (Scheme 1).

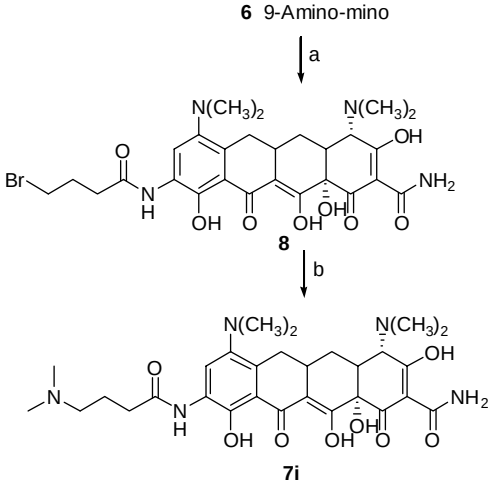
Treatment of 9-amino-mino **6** with 4-bromobutyrylchloride gave the intermediate **8**. Compound **8** was then treated with excess dimethylamine to give **7i** (Scheme 2). This compound was designed to probe the spacing requirement between the carbonyl and the nitrogen of the glycyl group. Compound **7h**, reported previously, was included for comparison.[9]

The 9-sulfonylamino derivatives **9a**–**e** were synthesized by treatment of 9-amino-mino **6** with appropriate sulfonyl chloride (Scheme 3). Final products were purified by HPLC.

The activities of the above new tetracycline derivatives were determined by the agar dilution method following



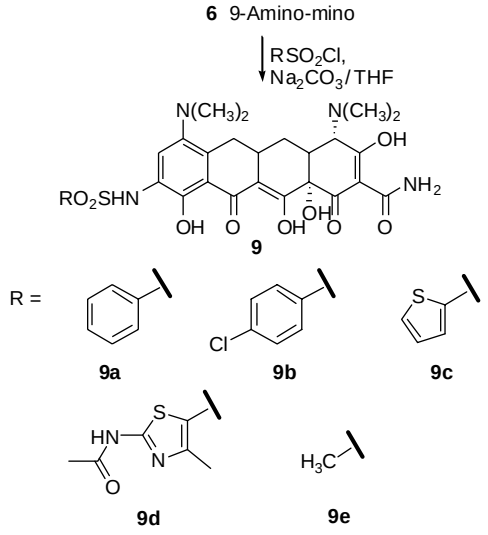
Scheme 1. Reagents and conditions: (a) HNO_3/H_2SO_4, 0 °C, 1 h; (b) 10% Pd/C, H_2, 40 psi.



Scheme 2. Reagents: (a) 4-bromobutyrylchloride/DMPU; (b) dimethylamine.

the recommendations of the National Committee for Clinical Laboratory Standards.[16] The arylacylamino derivatives in general show good activity against Gram-positive bacteria, with the best compound in this series **7e** exhibited MICs of 0.5–1.0 μg/mL. However, this series was less active against Gram-negative bacteria; most of the MICs are greater than the highest concentration tested (>32 μg/mL) (Table 1).

Compound **7g**, designed with a methylene in place of NH, is less active than **7h**, suggesting that the basicity



Scheme 3. Sulfonylamino derivatives of minocycline.

of cyclopentylamine is crucial for the potent broad-spectrum antibacterial activity (Table 2). Extension of the carbon linker between the glycyl dimethylamino nitrogen and the carbonyl, for example compound **7i**, led to reduction in overall activity when compared to that of the dimethylglycylamido derivative (DMG-MINO) **3**, indicating that the spacing between the carbonyl and the nitrogen is crucial for binding to the protein, and that gylcyl moiety seems to be the optimal group for the antibacterial potency in both Gram-positive and Gram-negative strains.

The sulfonamide derivatives show the most interesting in vitro activity. Almost all the compounds listed in Table 3 exhibited potent activity against Gram-positive pathogens and were considerably less active against Gram-negative bacteria. Compounds **9b** and **e** show very good activity against *Streptococcus. aureus* strain carrying the *tet*M-resistant determinant (MIC 0.06–0.25 μg/mL). In general, these compounds with MICs of 1 and 8 μg/mL, respectively, were moderately active against the *S. aureus* strain carrying the *tet*K-resistant determinant (efflux). The best compound in sulfonamide series is **9b**, demonstrating good activity with MICs against Gram-positive bacteria ranging from 0.06 to 1.0 μg/mL. The fact that the sulfonamide and the acylamido derivatives are much less active against Gram-negative bacteria than the glycylcyclines[9] suggests that in addition to possibly structural requirements, the basicity of the cyclopentylamino group of **7h**, dimethylamino side chain in **3** (DMG-MINO), and *tert*-butylamino group in **4** (tigecycline) at the 9-glycylamido side chain is most likely responsible for the good activity against *Escherichia coli*. The poor activities against *E. coli* that are still sensitive to minocycline shown by acylamido derivatives **7a**–**g**,**j** and sulfonamide derivatives **9a**–**d** suggest that these compounds most likely are not being permeated into the Gram-negative cell wall. The moderate activity (MIC 8 μg/mL) of **9e** against *E. coli* carrying the *tet*B determinant suggest that it is possible for the compound to be able to gain entry into the bacteria cell and is most likely to be pumped out by the efflux protein.

Table 1. Antibacterial activity of 9-(arylacylamino)-7-(substituted)-6-demethyl-6-deoxytetracycline **7a**–**f**

Organism	Minimal inhibitory concentration (MIC) μg/mL compound					
	7a	**7b**	**7c**	**7d**	**7e**	**7f**
S. aureus UBMS 90-1 (*tet*M)	16	4	4	8	1	4
S. aureus UBMS 90-2 (*tet*M)	8	4	2	2	1	4
S. aureus UBMS 90-3 (sensitive)	8	1	1	1	0.5	2
S. aureus UBMS 88-7 (*tet*K)	16	8	8	4	1	32
S. aureus Smith (sensitive)	4	1	1	1	1	2
E. faecalis ATCC 29212	8	4	4	2	1	8
E. coli UBMS 88-1 (*tet*B)	>32	>32	>32	2	>32	>32
E. coli 88-2 (sensitive)	>32	>32	>32	>32	>32	>32
E. coli UBMS 89-1 (*tet*M)	>32	NT	NT	NT	>32	>32
E. coli 89-2 (sensitive)	>32	>32	>32	>32	>32	>32

Table 2. Antibacterial activity of 9-(alkylacylamino)-7-(substituted)-6-demethyl-6-deoxytetracycline **7g**–**j**

Organism	Minimal inhibitory concentration (MIC) μg/mL compound					
	7g	**7h**	**7i**	**7j**	DMG-MINO	GAR-936
S. aureus UBMS 90-1 (*tet*M)	8	0.25	8	4	0.12	0.12
S. aureus UBMS 90-2 (*tet*M)	8	NT	4	2	NT	NT
S. aureus UBMS 90-3 (sensitive)	4	0.12	2	1	0.06	0.25
S. aureus UBMS 88-7 (*tet*K)	8	2	16	>32	0.5	0.5
S. aureus Smith (sensitive)	4	0.25	2	0.5	0.06	0.25
E. faecalis ATCC 29212	8	0.12	4	32	0.06	0.12
E. coli UBMS 88-1 (*tet*B)	>128	0.25	>32	>32	0.25	0.5
E. coli UBMS 90-4 (*tet*M)	>128	0.25	16	>32	0.25	0.12
E. coli 90-5 (sensitive)	>128	0.25	16	>32	0.12	0.25

Table 3. Antibacterial activity of 9-(sulfonylamino)-7-(substituted)-6-demethyl-6-deoxytetracycline **9a–e**

Organism	Minimal inhibitory concentration (MIC) μg/mL compound					
	9a	**9b**	**9c**	**9d**	**9e**	Mino
S. aureus UBMS 90-1 (*tet*M)	4	0.25	1	32	0.25	8
S. aureus UBMS 90-2 (*tet*M)	0.06	0.12	0.25	4	0.06	2
S. aureus UBMS 90-3 (sensitive)	4	0.12	0.25	2	0.12	0.03
S. aureus UBMS 88-7 (*tet*K)	4	1	2	32	8	0.25
S. aureus Smith (sensitive)	4	0.12	0.03	2	0.12	0.06
E. faecalis ATCC 29212	0.06	0.06	0.25	1	0.06	2
E. coli UBMS 88-1 (*tet*B)	16	>32	32	>32	8	16
E. coli 88-2 (sensitive)	8	>32	8	>32	2	0.25
E. coli UBMS 89-1 (*tet*M)	ND	ND	ND	32	ND	>32
E. coli 89-2 (sensitive)	16	>32	16	>32	2	2

In summary, the sulfonamide series is much more active against Gram-positive bacteria than the acylated series. The structure–activity relationship studies of these compounds provided useful information on the structural requirements for activity against Gram-negative bacteria. It also indicated that it is possible to design compounds with activity selectively just against Gram-positive bacteria. The potent in vitro activity of some of the sulfonamide derivatives (e.g., **9b**,**e**) against resistant Gram-positive bacteria makes them potential candidates for the development of new antibiotics targeting selectively just the Gram-positive pathogens.

Acknowledgments

We thank Ms. Nilda Jacobus and Dr. Frank Tally for their support and the analytical chemistry group for analytical HPLC and spectral data. We also thank Dr. Jerauld Skotnicki and Dr. Tarek Mansour for helpful discussion during the preparation of the manuscript.

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