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Antibacterial Activity of Novel 7,9-Disubstituted Tetracycline Analogs

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Abstract

Background: The Tetraphase total synthesis approach can access tetracycline analogs that are inaccessible or difficult to access by traditional semisynthetic methods. Novel 7,9-disubstituted tetracyclines were designed and synthesized using this approach. These analogs were evaluated for antibacterial activity, and structure-activity relationships (SAR) were studied.

Method: Novel 7-fluoro-, 7-methoxy-, 7-trifluoromethoxy-, 7-trifluoromethyl- and 7-cyano-9-substituted tetracycline analogs were synthesized from D-ring and AB-ring precursors *via* a tandem Michael-Dieckmann annulation. The new analogs' *in vitro* antibacterial activities were evaluated by susceptibility testing according to CLSI guidelines. Testing included strains expressing the ribosomal protection gene, *tet(M)*, or efflux pump genes, *tet(K)* or *tet(A)*. *In vivo* efficacy was assessed in a mouse septicemia model against *Staphylococcus aureus* ATCC 13709.

Results: Antibacterial activity of representative 7,9-disubstituted tetracycline analogs

Compound	MIC (µg/mL)										PD ₅₀ mg/kg IV
	SA100 ATCC13709	SA161 MRSA, <i>tet(M)</i>	SA158 <i>tet(K)</i>	EF159 <i>tet(M)</i>	SP160 <i>tet(M)</i>	EC155 <i>tet(A)</i>	AB110 ATCC19606	PA111 ATCC27853	EC108 ATCC13047	KP153 <i>tet(A)</i>	
TP-902	0.13	0.5	0.25	0.5	0.25	> 32	1	> 32	16	> 32	4.3
TP-322	0.13	0.063	0.063	0.031	≤ 0.016	4	0.13	16	2	8	0.35
TP-175	0.25	0.13	0.25	0.063	0.031	4	0.5	16	2	4	0.36
TP-286	0.13	0.25	0.5	0.25	≤ 0.016	8	0.25	16	2	8	0.25
TP-336	0.031	0.13	0.063	0.031	≤ 0.016	4	0.063	16	2	8	1.4
TP-946	0.25	0.5	0.25	0.5	0.25	> 32	1	> 32	> 32	> 32	> 10

SA: *S. aureus*; EF: *E. faecalis*; SP: *S. pneumoniae*; EC: *E. coli*; AB: *A. baumannii*; PA: *P. aeruginosa*; EC1: *E. cloacae*; KP: *K. pneumoniae*.

Conclusions: Novel 7,9-disubstituted tetracycline analogs have potent antibacterial activities against Gram-positive and Gram-negative organisms including tetracycline-resistant strains. A number of analogs also displayed potent *in vivo* efficacy in a mouse septicemia infection model. These results demonstrate that the Tetraphase total synthesis approach can access novel and potent tetracycline analogs that are inaccessible, or difficult to access, by traditional semisynthetic methods.

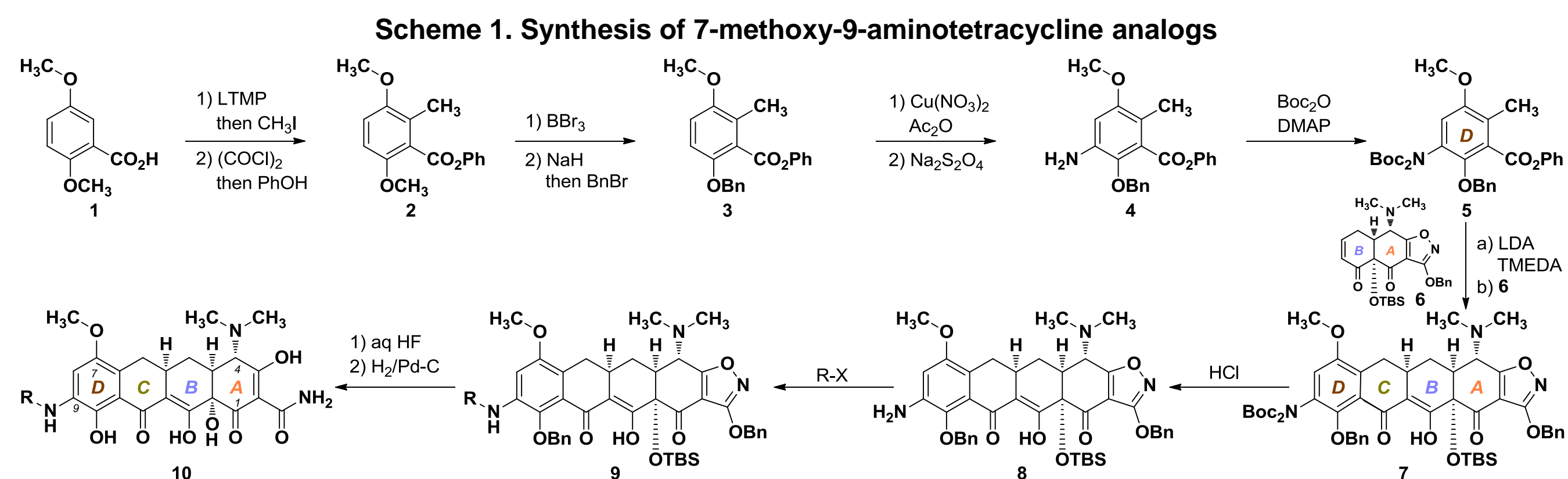
Methods

Bacterial Strains. Strains with defined tetracycline resistance mechanisms were obtained from M. Roberts (University of Washington, Seattle, WA). Other strains were from the American Type Culture Collection (ATCC), Micromyx (Kalamazoo, MI; *S. aureus* SA161), or Clinical Microbiology Institute (Wilsonville, OR).

***In vitro* Susceptibility.** Compounds were dissolved in water and assayed in microtiter plates according to CLSI methodology.¹

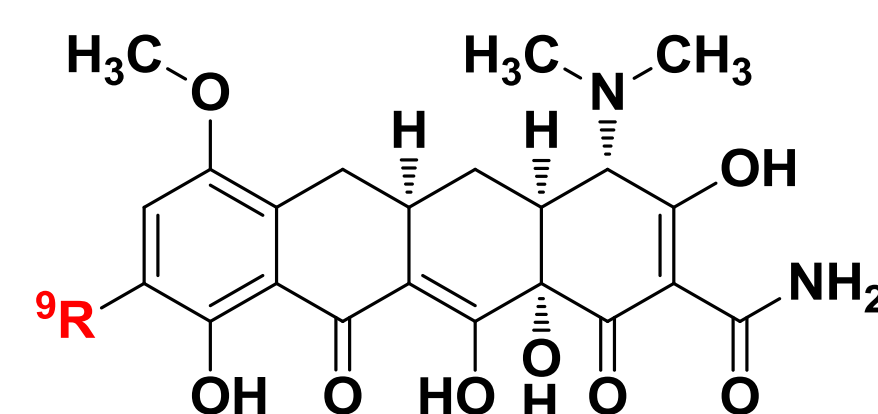
Mouse Systemic Infection Studies. Mice received treatment *via* intravenous (IV) injection 1 hour post intraperitoneal (IP) infection. At termination of study (48 hrs post-dose), percent survival was calculated and the dose (mg/kg) affecting 50% survival, the protective dose 50% (PD₅₀), was reported along with 95% confidence intervals as calculated by Probit analysis using GraphPad Prism version 4.03 (GraphPad Software).

Materials. 7,9-Disubstituted tetracycline analogs were synthesized from appropriate D-ring precursors (such as **5**) and the bicyclic enone **6**² *via* a Michael-Dieckmann annulation. A typical synthesis of the 7-methoxy-9-aminotetracycline analogs is shown in Scheme 1.



Results

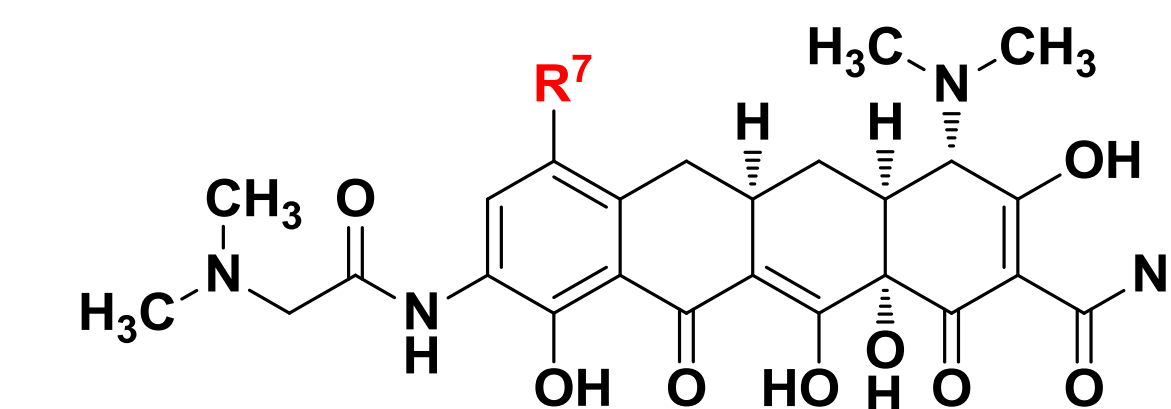
Table 1. *In vitro* antibacterial activity of 7-methoxy-9-R-tetracycline analogs



Compound	R ⁷	MIC (µg/mL)									
		SA100 ATCC13709	SA161 MRSA, <i>tet(M)</i>	SA158 <i>tet(K)</i>	EF159 <i>tet(M)</i>	SP160 <i>tet(M)</i>	EC155 <i>tet(A)</i>	AB110 ATCC19606	PA111 ATCC27853	EC108 ATCC13047	KP153 <i>tet(A)</i>
TP-508	H ₃ C	0.5	8	1	8	16	32	0.13	32	8	32
TP-628	H ₃ C-CH ₂ -N(CH ₃) ₂	0.5	0.5	0.063	0.25	≤ 0.016	2	1	32	1	4
TP-307	H ₃ C-CH ₂ -N(CH ₃) ₂	32	> 32	16	> 32	32	> 32	> 32	> 32	> 32	> 32
TP-644	H ₃ C-CH ₂ -O-CH ₂ -N(CH ₃) ₂	1	1	2	2	2	> 32	> 32	> 32	> 32	> 32
TP-197	H ₃ C-CH ₂ -CH ₂ -N(CH ₃) ₂	2	2	2	4	2	> 32	> 32	> 32	> 32	> 32
TP-698	Phenyl	2	4	2	4	4	> 32	8	> 32	> 32	> 32
TP-286	H ₃ C-CH ₂ -N(CH ₃) ₂	0.13	0.25	0.5	0.25	≤ 0.016	8	0.25	16	2	8
TP-175	H ₃ C-CH ₂ -N(CH ₃) ₂	0.25	0.13	0.25	0.063	0.031	4	0.5	16	2	4
TP-322	H ₃ C-CH ₂ -N(CH ₃) ₂	0.13	0.063	0.063	0.031	≤ 0.016	4	0.13	16	2	8
TP-690	H ₃ C-CH ₂ -N(CH ₃) ₂	1	2	1	1	0.031	8	1	> 32	4	16
TP-902	H ₃ C-CH ₂ -N(CH ₃) ₂	0.13	0.5	0.25	0.5	0.25	> 32	1	> 32	16	> 32
TP-946	H ₃ C-CH ₂ -N(CH ₃) ₂	0.25	0.5	0.25	0.5	0.25	> 32	1	> 32	> 32	> 32
TP-930	H ₃ C-CH ₂ -N(CH ₃) ₂	0.5	8	0.5	0.5	0.13	16	2	> 32	4	8
TP-406	H ₃ C-CH ₂ -N(CH ₃) ₂	1	0.5	0.5	1	8	> 32	> 32	> 32	> 32	> 32
TP-312	H ₃ C-CH ₂ -N(CH ₃) ₂	4	> 32	16	32	8	> 32	> 32	> 32	> 32	> 32
tetracycline		0.5	32	>32	>32	>32	>32	1	32	1	32
tigecycline		0.063	0.13	0.13	0.063	≤ 0.016	1	0.5	16	0.25	1

Results

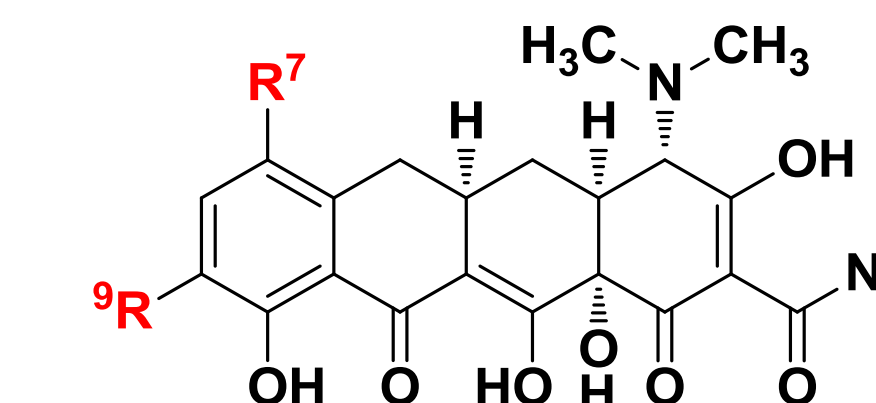
Table 2. *In vitro* antibacterial activity of 7-R-9-dimethylaminoacetamidotetracycline analogs



Compound	R ⁷	MIC (µg/mL)									
		SA100 ATCC13709	SA161 MRSA, <i>tet(M)</i>	SA158 <i>tet(K)</i>	EF159 <i>tet(M)</i>	SP160 <i>tet(M)</i>	EC155 <i>tet(A)</i>	AB110 ATCC19606	PA111 ATCC27853	EC108 ATCC13047	KP153 <i>tet(A)</i>
TP-286	CH ₃ O	0.13	0.25	0.5	0.25	≤ 0.016	8	0.25	16	2	8
TP-550	CF ₃ O	≤ 0.016	0.13	0.5	0.031	≤ 0.016	8	≤ 0.016	16	2	ND
TP-535 ⁽⁹⁾	F	0.5	0.13	0.25	0.031	≤ 0.016	8	0.13	8	1	8
TP-514	CF ₃	0.25	0.25	2	0.063	≤ 0.016	32	0.5	32	8	32
TP-999	CN	1	2	8	2	0.5	> 32	> 32	> 32	16	> 32

Table 3. *In vivo* antibacterial activity of 7,9-disubstituted tetracycline analogs

Compound	R ⁷	R ⁹	MIC (µg/mL)	PD ₅₀
			SA100 ATCC13709	mg/kg IV
TP-286	CH ₃ O	H ₃ C-CH ₂ -N(CH ₃) ₂	0.13	0.25
TP-175	CH ₃ O	H ₃ C-CH ₂ -N(CH ₃) ₂	0.25	0.36
TP-322	CH ₃ O	H ₃ C-CH ₂ -N(CH ₃) ₂	0.13	0.35
TP-902	CH ₃ O	H ₃ C-CH ₂ -N(CH ₃) ₂	0.13	4.3
TP-946	CH ₃ O	H ₃ C-CH ₂ -N(CH ₃) ₂	0.25	>10
TP-336	CF ₃ O	H ₃ C-CH ₂ -N(CH ₃) ₂	0.031	1.4



Conclusions

- A set of new substituents were incorporated into the tetracycline scaffold at the C7 and C9 positions using the Tetraphase total synthesis approach. This led to a series of novel 7,9-disubstituted tetracycline analogs with increased structural diversity and the potential to overcome tetracycline resistance.
- All C7 substituents investigated, except CN, are tolerated and result in potent tetracycline analogs. On the other hand, aminoacylamido groups are preferred at C9 for optimal antibacterial activity.
- A number of new analogs, especially TP-286, TP-175, and TP-322, are highly potent against a broad range of Gram-positive and Gram-negative pathogens *in vitro* and *in vivo*.

References & Notes

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