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F1-1856

51st Annual ICAAC
17-20 September, 2011
Chicago, IL

Antibacterial Activity of Novel 8-Aminomethyl Substituted Tetracyclines

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Abstract

Background: Total synthesis of tetracyclines allows access to chemical space that is inaccessible by traditional semisynthetic methods. In particular, the C-8 position has been largely unexplored, yet is easily modified via the Tetraphase total synthetic platform. The unique *in vitro* and *in vivo* profiles of a series of 8-aminomethyl tetracycline analogs were studied.

Method: Novel compounds were synthesized via a tandem Michael-Dieckmann reaction with a D-ring precursor. *In vitro* antibacterial activities were evaluated according to CLSI guidelines. Efficacy following oral administration was determined in mouse kidney infection models challenged with uropathogenic *E. coli* or *K. pneumoniae* and in a neutropenic mouse lung model challenged with *tet(M)* MRSA SA191 (VL-137).

Results: Modifications at the C-7 and C-8 positions initially led to compounds with good *in vitro* activity for bacterial pathogens expressing either *tet(M)* (TP-333) or *tet(A)* (TP-837 and TP-8060). Combining structural information from these two subsets yielded TP-4622, a compound with balanced activity against both types of tetracycline-resistant bacteria. The latter 3 compounds exhibited efficacy in two murine urinary tract infection models following oral administration. TP-4622 was also orally efficacious in a murine lung infection model.

Cmpd	MIC (µg/mL)								log ₁₀ CFU/g reduction		
	SA100 ATCC 13709	SA161 MRSA <i>tet(M)</i>	SA158 <i>tet(K)</i>	SP160 <i>tet(M)</i>	EC107 ATCC 25922	EC133 ESβL	KP153 <i>tet(A)</i>	KP457 ESβL	EC200 (ESβL) 2 mg/kg	KP453 (ESβL) 50 mg/kg	VL-137 (MRSA) 50 mg/kg
TP-333	0.25	0.25	0.25	1	1	ND	8	ND	ND	ND	ND
TP-837	0.13	8	0.063	1	0.13	0.13	0.5	2	2.78	2.15	ND
TP-8060	0.25	8	0.13	0.5	0.063	0.25	1	0.5	2.99	2.93	ND
TP-4622	0.13	2	0.13	0.25	0.13	0.25	0.5	1	2.30	2.16	1.98
Tetracycline	0.5	32	>32	>32	1	>32	>32	4	ND	1.10	0.09
Levofloxacin	0.13	16	0.25	1	0.016	>32	2	64	3.78	1.17	2.27

SA: *Staphylococcus aureus*; SP: *Streptococcus pneumoniae*; EC: *Escherichia coli*; EF: *Enterococcus faecalis*;
KP: *Klebsiella pneumoniae*; MRSA=methicillin-resistant *S. aureus*; ESβL = extended spectrum β-lactamase; ND: Not done

Conclusions: The total synthetic approach allows access to novel tetracyclines that cannot be made by traditional semisynthesis. 8-Aminomethyl substituted tetracyclines can be tuned for activity against pathogens expressing known tetracycline resistance genes and have exhibited significant oral activity in clinically relevant murine models of infection.

Methods

Chemistry synthesis. A representative synthesis of the 8-aminomethyltetracyclines is shown in Figure 1. See Patent Application WO 2010/129057 for additional details.

In vitro susceptibility. Compounds were dissolved in water and assayed according to CLSI methodology. Strains with defined tetracycline-resistant mechanisms were obtained from M. Roberts (Univ. 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).

Oral efficacy screen. CD-1 female mice (18-22 g) were intraperitoneally injected with 0.5 ml *S. aureus* ATCC 13709 (1-2 x 10⁶ cfu/mouse) diluted in 5% hog gastric mucin, routinely achieving ≥90% mortality at 48 hrs. One hour post-challenge, mice (n=6 per group) were administered compound at 3 mg/kg intravenously or 30 mg/kg orally. At 48 hours post-dose, percent survival was recorded.

MRSA lung model. Female BALB/c mice (18 to 20 g) were pre-treated with cyclophosphamide to render the mice neutropenic. Mice were infected with 0.5 mL MRSA SA191 via intranasal administration. At 2 and 12 hours post infection mice (6 per group) were treated orally with test article (50 mg/kg) or linezolid (30 mg/kg). At 24 hours post initiation of treatment mice were euthanized by CO₂ inhalation. Lungs were removed, weighed, homogenized, serially diluted, and plated on TSA medium. The plates were incubated overnight at 37 °C in 5% CO₂. CFU/gram of lung was calculated by enumerating the plated colonies then adjusting for serial dilutions and lung weight.

Kidney infection models. Female BALB/c mice (18 to 20 g) were infected with 0.2 mL *E. coli* EC200 or *K. pneumoniae* KP453 via intravenous injection. At 12 and 24 hours post infection mice (6 per group) were treated orally with test article. At 36 hours post initiation of treatment mice were euthanized by CO₂ inhalation. The kidneys were removed, weighed, homogenized, serially diluted, and plated on TSA medium. The plates were incubated overnight at 37°C in 5% CO₂. CFU/gram of kidney was calculated by enumerating the plated colonies then adjusting for serial dilution and kidney weight.

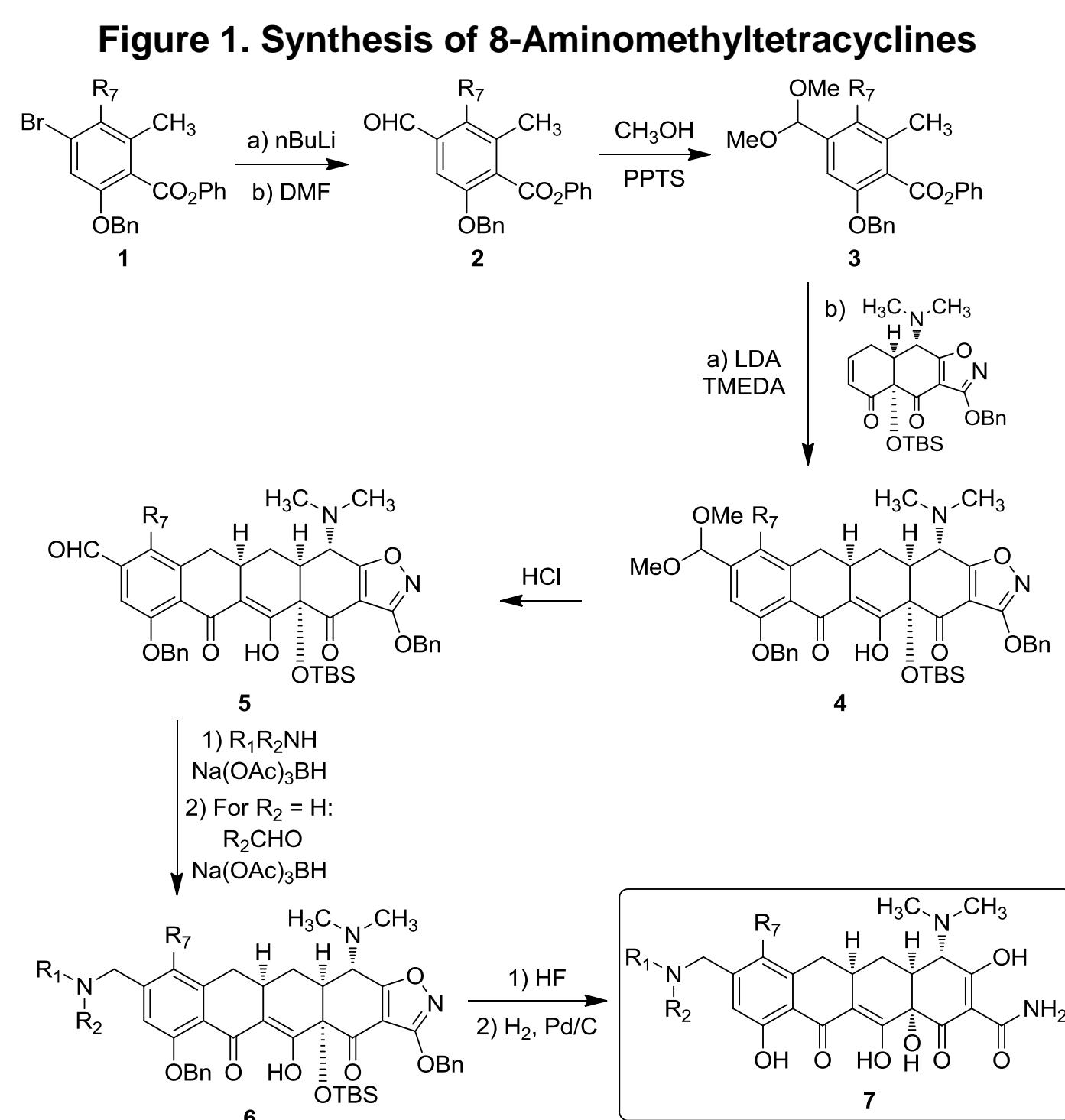


Table 1. Antibacterial activity of 8-aminomethyltetracyclines. Small, α-branched secondary amines or cyclic amines give good Gram-negative activity, including *tet(A)*, but have poor *tet(M)* activity. Compounds are orally efficacious in a murine septicemia screen.

TP #	R ₇	R ₁ -N(R ₂)	MIC (µg/mL)								S. aureus Sepsis (% Survival)		
			SA101 ATCC 29213	SA161 MRSA <i>tet(M)</i>	SA158 <i>tet(K)</i>	EF159 <i>tet(M)</i>	SP106 ATCC 49619	SP160 <i>tet(M)</i>	EC107 ATCC 25922	EC155 <i>tet(A)</i>	KP153 <i>tet(A)</i>	IV 3 mg/kg	PO 30 mg/kg
TP-574	F		0.25	32	0.125	16	0.313	1	0.25	8	4	ND	ND
TP-907	F		0.25	8	0.125	16	0.313	1	0.25	2	2	ND	ND
TP-837	F		0.125	8	0.063	16	0.016	1	0.125	0.5	0.5	100%	100%
TP-903	F		0.063	4	0.063	8	0.016	0.5	0.125	0.5	0.5	100%	100%
TP-954	Cl		0.016	4	0.031	4	0.016	1	0.031	0.5	0.25	100%	100%
TP-2212	Cl		0.063	16	0.031	8	0.016	1	0.031	1	1	100%	100%
TP-899	(CH ₃) ₂ N		0.125	8	0.125	8	0.031	1	0.125	0.5	0.5	100%	100%
TP-5536	(CH ₃) ₂ N		0.5	16	0.125	16	≤0.016	1	0.125	0.5	0.5	100%	100%
TP-8060	OCH ₃		0.25	8	0.125	8	≤0.016	0.5	0.063	1	1	100%	100%
TP-1145	CF ₃		≤0.016	8	≤0.016	8	≤0.016	0.5	≤0.016	1	1	100%	100%
Tetracycline			0.5	32	>32	>32	0.25	>32	1	>32	32	100%	100%

SA: *Staphylococcus aureus*; SP: *Streptococcus pneumoniae*; EC: *Escherichia coli*; EF: *Enterococcus faecalis*; KP: *Klebsiella pneumoniae*; ND: Not done

Table 2. Antibacterial activity. More lipophilic, branched amines or less basic amines give good *tet(M)* activity but have reduced Gram-negative activity. Lower oral and IV efficacy is observed.

TP #	R ₇	R ₁ -N(R ₂)	MIC (µg/mL)								S. aureus Sepsis (% Survival)		
			SA101 ATCC 29213	SA161 MRSA <i>tet(M)</i>	SA158 <i>tet(K)</i>	EF159 <i>tet(M)</i>	SP106 ATCC 49619	SP160 <i>tet(M)</i>	EC107 ATCC 25922	EC155 <i>tet(A)</i>	KP153 <i>tet(A)</i>	IV 3 mg/kg	PO 30 mg/kg
TP-828	F		0.125	0.125	0.063	0.125	0.125	1	2	4	8	33%	0%
TP-386	F		0.125	0.5	0.125	0.5	0.063	1	1	4	8	33%	ND
TP-072	F		0.25	0.5	0.125	0.5	0.25	2	0.5	4	4	17%	25%
TP-102	F		0.25	0.5	0.25	0.5	0.125	1	1	4	8	33%	0%
TP-3373	Cl		0.125	1	0.031	1	0.016	1	0.25	4	4	100%	83%
TP-6615	Cl		0.25	1	0.016	1	0.016	2	1	16	32	100%	67%

Results

Table 3. Antibacterial activity. Combining α-branching and more lipophilic substituents or larger cyclic amines gives balanced activity. Oral activity is maintained.

TP #	R ₇	R ₁ -N(R ₂)	MIC (µg/mL)								S. aureus Sepsis (% Survival)		
			SA101 ATCC 29213	SA161 MRSA <i>tet(M)</i>	SA158 <i>tet(K)</i>	EF159 <i>tet(M)</i>	SP106 ATCC 49619	SP160 <i>tet(M)</i>	EC107 ATCC 25922	EC155 <i>tet(A)</i>	KP153 <i>tet(A)</i>	IV 3 mg/kg	PO 30 mg/kg
TP-158	F		0.063	2	0.031	2	0.031	0.5	0.031	1	1	100%	100%
TP-313	F		0.25	0.125	0.125	2	0.016	1	0.5	2	2	50%	75%
TP-6271	F		0.25	2	0.25	2	≤0.016	0.5	0.25	2	2	83%	67%
TP-9931	Cl		0.063	2	0.016	1	0.016	0.5	0.016	0.5	0.5	100%	100%
TP-6812	Cl		0.125	1	0.063	1	0.016	1	0.125	1	1	100%	100%
TP-4622	OCH ₃		0.125	2	0.125	4	0.016	0.25	0.125	0.5	0.5	100%	100%
TP-3224	OCH ₃		0.25	2	0.063	4	≤0.016	0.25	0.063	0.25	0.5	100%	100%

Table 4. MIC₉₀ profiles, MICs of isolates used in efficacy models, and oral activity in murine lung (SA191) and kidney (EC200, KP453) infection models.

Compound	MIC ₉₀ (µg/mL)			Infection Model Isolates MIC (µg/mL)			Murine Infection Models (log ₁₀ CFU/g reduction)		
	<i>S. aureus</i> (MRSA, n = 13)	<i>E. coli</i> (ESβL, n = 14)	<i>K. pneumoniae</i> (n = 19)	SA191 VL-137 (MRSA)	EC200 (ESβL)	KP453 (ESβL)	SA191 (MRSA) 50 mg/kg	EC200 (ESβL) 2 mg/kg	KP453 (ESβL) 50 mg/kg
TP-837	ND	0.5	1	ND	0.25	2	ND	2.78	2.15
TP-8060	4	1	1	ND	0.5	0.5	ND	2.99	2.93
TP-4622	2	0.5	2	1	0.25	1	1.98	2.30	2.16
Tetracycline	>32	>32	>64	>64	>32	4	0.09	ND	1.10
Linezolid	4	ND	ND	2	ND	ND	2.26 (30 mg/kg)	ND	ND
Levofloxacin	>32	32	64	0.25	0.063	32	1.97	3.78	1.17

Conclusions

- The Tetraphase total synthetic platform allows access to novel tetracyclines, yielding increased chemical diversity in the class and providing compounds that overcome bacterial resistance.
- 8-Aminomethyl substituted tetracyclines can be tuned for activity against pathogens expressing known tetracycline resistance genes as well as for oral efficacy.
- Compounds exhibited oral activity in clinically relevant murine models of infection that was comparable to standard-of-care antibiotics.