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Fluorocyclines: A New Class of Broad-Spectrum Tetracyclines with Antibacterial Properties

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Abstract

Background: Total synthesis of tetracyclines allows access to chemical space that is inaccessible by traditional semisynthetic methods. Fluorine, in particular, poses challenges for tetracycline semisynthesis, yet is readily introduced *via* the current platform. The unique *in vitro* and *in vivo* profiles of these fluorocyclines were studied.
Method: Novel compounds were synthesized *via* a tandem Michael-Dieckmann reaction with a key enone and a D-ring precursor, followed by modifications after C-ring annulation. *In vitro* antibacterial activities were evaluated according to CLSI guidelines. Efficacy following oral or intravenous administration was determined in a mouse systemic infection model challenged with *Staphylococcus aureus* ATCC 13709.
Results: *In vitro* activity for bacterial pathogens expressing efflux and ribosomal protection tetracycline-resistant genes can be modulated.

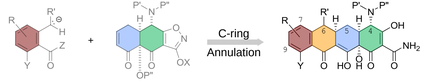
Cmpd	MIC (μg/mL)										PD50	
	SA100	SA161	SA158	SP106	SP160	EC107	EC155	AB110	KP153		IV	PO
	ATCC 13709	MRSA *tet*(M)	*tet*(K)	ATCC 49619	*tet*(M)	ATCC 25922	*tet*(A)	ATCC 19606	*tet*(A)		(mg/kg)	(mg/kg)
TP-043	0.25	2	16	0.031	0.5	1	>32	4	>32		1.2	>30
TP-493	0.13	0.25	4	0.016	0.063	0.25	>32	1	>32		1.4	>30
TP-594	0.13	0.5	0.063	0.016	0.016	2	>32	0.5	>32		ND	ND
TP-170	0.063	0.063	0.063	0.031	0.031	0.13	4	0.063	2		0.43	14
TP-221	0.13	0.25	0.063	0.016	0.016	0.25	2	0.5	2		0.3	ND
Tetracyline	0.5	32	>32	0.25	>32	1	>32	1	>32		1	6.9

SA: *S. aureus*; SP: *Streptococcus pneumoniae*; EC: *Escherichia coli*; AB: *Acinetobacter baumannii*; KP: *Klebsiella pneumoniae*; MRSA=methicillin-resistant *S. aureus*; ND: Not done

Conclusion: Novel fluorocycline analogs are readily prepared by total synthesis, allowing for rapid optimization of their pharmacological properties and advancement into development.

Introduction

- The total synthetic approach discovered in Professor Andrew Myers' lab (Charest, MG, et al. *Science* **2005**, *308*, 395-398) has reinvigorated the field of tetracycline chemistry
- Convergent synthesis, adequate sourcing and process research enables total synthesis of novel tetracyclines to be cost competitive
- Small changes to the tetracyclic core can profoundly affect spectrum, ability to evade resistance mechanisms, and pharmacokinetic properties

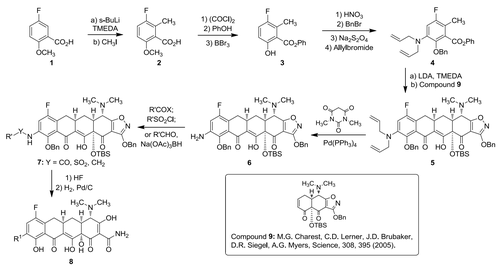


Left Hand Side | Key Enone | Novel Analogs

Methods

Chemistry synthesis. A typical synthesis of a fluorocycline is shown in Figure 1.
Bacterial Strains. 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) or Micromyx (Kalamazoo, MI; *S. aureus* SA161).
***In vitro* susceptibility.** Compounds were dissolved in water and assayed in microtiter plates according to CLSI standards.
***In vivo* screen.** CD-1 female mice (18-22 g) were intraperitoneally injected with 0.5 ml of *Staphylococcus aureus* ATCC 13709 (1-2 x 10^6 cfu/mouse) diluted in 5% hog gastric mucin, an inoculum that routinely achieved ≥90% mortality after 48 hrs. At one hour post-challenge, mice (n=6 per group) were administered compound at concentrations ranging from 10 to 0.05 mg/kg intravenously or 30 to 0.3 mg/kg orally. Forty-eight hours post-dose, percent survival was recorded, and the dose that protected 50% of the mice from death (PD_{50} in mg/kg) was calculated.

Figure 1. Synthetic Scheme for Fluorocyclines



Compound **9**: M.G. Charest, C.D. Lerner, J.D. Brubaker, D.R. Siegel, A.G. Myers, Science, 308, 395 (2005).

Results

Table 1. Antibacterial Activity of Fluorocyclines with Cyclic Amino Acid Side-Chains

TP #	Compound **8** R^1 =	MICs (μg/mL)													PD50 (mg/kg)	
		SA100	SA161	SA158	EF103	EF159	SP106	SP160	EC107	EC155	AB110	PA111	KP109	KP153	SA100	
		ATCC 13709	MRSA *tet*(M)	*tet*(K)	ATCC 29212	*tet*(M)	ATCC 49619	*tet*(M)	ATCC 25922	*tet*(A)	ATCC 19606	ATCC 27853	ATCC 13883	*tet*(A)	IV	PO
TP-221		0.25	0.25	0.063	0.063	0.125	0.016	0.016	0.25	2	0.5	16	1	2	0.3	ND
TP-434		0.063	0.063	0.063	0.016	0.016	0.016	0.016	0.063	1	0.125	8	0.5	0.5	0.3	
TP-170		0.063	0.063	0.063	0.016	0.016	0.031	0.031	0.125	4	0.063	4	0.063	2	0.43	14
TP-271		0.125	0.031	≤0.016	0.063	≤0.016	≤0.016	≤0.016	0.063	4	0.063	32	2	4		
TP-740		2	2	2	1	2	0.25	0.25	2	16	1	>32	4	16		
TP-645		2	8	1	1	1	0.125	0.25	0.5	>32	1	>32	2	>32		
Tetracycline		0.5	32	>32	16	>32	0.25	>32	1	>32	1	>32	2	32	1	6.9

Table 2. Antibacterial Activity of 9-Arylamide-Fluorocyclines

TP #	Compound **8** R^1 =	MICs (μg/mL)													PD50 (mg/kg)	
		SA100	SA161	SA158	EF103	EF159	SP106	SP160	EC107	EC155	AB110	PA111	KP109	KP153	SA100	
		ATCC 13709	MRSA *tet*(M)	*tet*(K)	ATCC 29212	*tet*(M)	ATCC 49619	*tet*(M)	ATCC 25922	*tet*(A)	ATCC 19606	ATCC 27853	ATCC 13883	*tet*(A)	IV	PO
TP-314		0.25	0.5	0.5	0.5	0.5	8	8	>32	>32	>32	>32	>32	>32		
TP-385		0.063	0.125	0.063	0.063	0.063	2	4	>32	>32	>32	>32	>32	>32		
TP-555		0.25	0.5	0.5	0.5	0.5	2	2	>32	>32	32	>32	>32	>32		
TP-515		0.5	0.5	1	1	1	1	2	>32	>32	>32	>32	>32	>32		
TP-278		0.125	0.125	0.125	0.125	0.125	4	8	>32	>32	>32	>32	>32	>32	>10	>30
TP-285		2	4	16	4	4	1	2	>32	>32	16	>32	>32	>32		
TP-616		0.25	0.25	0.5	0.25	1	16	16	>32	>32	>32	>32	>32	>32		
TP-886		0.063	0.063	0.063	0.063	0.125	2	8	>32	>32	>32	>32	>32	>32		

Results

Table 3. Antibacterial Activity of Fluorocyclines with Amide Bond Replacements

TP #	Compound **8** R^1 =	MICs (μg/mL)													PD50 (mg/kg)	
		SA100	SA161	SA158	EF103	EF159	SP106	SP160	EC107	EC155	AB110	PA111	KP109	KP153	SA100	
		ATCC 13709	MRSA *tet*(M)	*tet*(K)	ATCC 29212	*tet*(M)	ATCC 49619	*tet*(M)	ATCC 25922	*tet*(A)	ATCC 19606	ATCC 27853	ATCC 13883	*tet*(A)	IV	PO
TP-863	H	0.25	2	1	2	2	0.25	4	1	16	0.016	16	4	8		
TP-034	NH_2	0.25	2	2	0.5	4	0.25	4	2	32	0.25	16	4	32		
TP-950		0.5	0.5	0.5	0.25	0.5	1	4	32	>32	2	>32	32	>32		
TP-589		0.25	2	0.25	1	1	4	16	>32	>32	>32	>32	>32	>32		
TP-162		1	4	1	1	4	0.125	0.25	2	16	32	>32	8	16		
TP-457		0.25	2	2	0.5	4	0.063	0.5	2	>32	0.5	>32	8	>32		
TP-043		0.25	2	16	0.5	16	0.03	0.5	1	>32	4	32	4	>32	1.2	>30
TP-493		0.125	0.25	4	0.125	0.5	0.016	0.06	0.25	>32	1	32	2	>32	1.4	>30
TP-663		0.5	1	0.5	0.5	1	0.25	0.25	32	>32	1	>32	>32	>32		
TP-665		0.5	1	0.5	0.5	2	0.25	1	>32	>32	1	>32	>32	>32		
TP-953		1	2	8	2	2	0.125	0.5	8	>32	32	>32	>32	>32		
TP-767		8	>32	8	8	32	1	4	>32	>32	>32	>32	>32	>32		
TP-153		2	4	2	1	2	0.25	0.25	4	16	2	>32	8	8		

SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; SP: *Streptococcus pneumoniae* : EC: *Esherichia coli*; AB: *Acinetobacter baumannii*; PA: *Pseudomonas aeruginosa*; KP: *Klebsiella pneumoniae*.

Conclusions

- **The totally synthetic platform is highly versatile, readily producing fluorocycline deriviatives with activity against pathogens expressing tetracycline-specific efflux and ribosomal protection genes**
- **Introduction of F at C7 coupled with a pyrrolidino at C9 provided optimal *in vitro* potency (posters F1-2157- F1-2160), *in vivo* efficacy (F1-2161-2164), and pharmacokinetics (posters A1-027, A1-028, F1-2163)**
- **TP-271 exhibited good *in vitro* and *in vivo* antibacterial activity against a wide range of community-acquired pathogens (See posters F1-2156, 2159, and 2160 for further profiling)**