SUN-332

TP-6076, A Fully Synthetic Tetracycline Antibacterial Agent, Is Highly Potent against a Broad Range of Pathogens, including Carbapenem-resistant *Enterobacteriaceae*

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Abstract

Background/Introduction: The fully synthetic tetracycline platform has enabled the systematic modification and structure-activity relationship studies of structurally diverse tetracyclines, such as analogs with novel substitutions at C4, C7, and C8, which are inaccessible or extremely difficult to access by semisynthetic methods. A number of the new tetracycline analogs displayed potent *in vitro* antibacterial activity against a broad range of pathogens, including carbapenem-resistant strains of *Klebsiella pneumoniae, Acinetobacter baumannii*, and *Escherichia coli*.

Methods: 4,7,8-Trisubstituted tetracycline analogs were synthesized from an *N,N*-diallyl AB-ring enone and various D-ring precursors. *In vitro* antibacterial activity was determined by susceptibility testing of bacteria strains, including strains expressing tetracycline-resistant ribosomal protection (*tet*(M)) or efflux (*tet*(A) or *tet*(K)) mechanisms, according to CLSI guidelines.

Compound #	MIC (μg/mL)																
	SA101	SA161	SA158	EF327	EF404	SP160	EC107	EC155	EC878	KP457	PM385	PA555	PA556	EC603	AB250	SM256	BC240
	29213	tetM	tetK	tetM	tetM	tetM	25922	tetA	tolC	CTX-M-15		BAA-47	PAO750	tetA			
1c	1	32	0.5	8	2	0.25	≤0.016	2	≤0.016	0.25	0.25	8	0.03	2	4	2	4
1d	1	32	0.25	8	2	0.25	≤0.016	2	0.03	0.5	0.5	8	0.12	8	4	2	8
TP-6076	0.03	0.5	0.03	0.12	0.03	≤0.016	≤0.016	≤0.016	≤0.016	0.03	0.12	4	0.03	0.5	0.12	0.06	1
1m	0.25	16	0.06	8	2	0.5	≤0.016	0.25	≤0.016	0.25	1	16	0.25	1	0.25	0.25	4
1q	1	32	0.25	8	2	0.25	≤0.016	4	≤0.016	0.25	1	16	0.06	16	8	4	8

Conclusions: Fully synthetic tetracycline analogs with novel substitutions at C4, C7, and C8 were designed and synthesized using the total synthesis approach, and their antibacterial activity-structure relationships were systematically studied. A number of these new tetracyclines displayed high *in vitro* potency against a broad range of clinically important pathogens, including carbapenem-resistant *Enterobacteriaceae* (CRE). TP-6076, a lead compound from this novel chemical series, is currently being evaluated in phase 1 clinical studies.

Methods

Clinical isolates. Recent clinical isolates possessing diverse demographic, genotypic and phenotypic characterizations were obtained from Eurofins Medinet (Chantilly, VA) or International Health Management Associates, Inc. (IHMA; Schaumburg, IL). Species-appropriate quality control (QC) strains were used to ensure laboratory standards as guided by CLSI[i] and the QC strains were obtained from the American Type Culture Collection (Manassas, VA). TP-6076 and comparators was tested against a set of *A. baumannii* strains with characterized *adeAB* expression[ii] and a set of well-characterized *K. pneumoniae* mutant strains to further evaluate the impact of *ramA* over-expression on susceptibility;[iii] both strain sets were obtained from Wyeth (now Pfizer).

Minimum inhibitory concentration (MIC) assays with clinical isolates were performed essentially as described by the Clinical Laboratory Standards Institute.[i] Resistant phenotypes were determined using CLSI breakpoints[iv] except for tigecycline, where FDA breakpoints were used.[v]

Susceptibility testing against *E. coli* DH10B expressing recombinant tetracycline-resistance genes. To determine the impact of specific tetracycline-resistance genes in isogenic *E. coli* strains, sequences encoding *tet*(A), *tet*(B), *tet*(D), *tet*(K), *tet*(M), *tet*(Q), *tet*(X) and *E. coli* β-galactosidase (*lacZ*) as a negative control were amplified by PCR from clinical isolates confirmed by prior sequencing to have these tetracycline-resistance determinants. Genes were cloned into an L-arabinose inducible expression system without any affinity tags (pBAD-Myc-His, Invitrogen, Carlsbad, CA). Plasmids were transformed into *E. coli* DH10B cells (Invitrogen, Carlsbad, CA). Cloned inserts were verified by sequencing and

Methods (cont'd)

comparing with reported sequences in GenBank (accession numbers: *tet*(A), AJ419171; *tet*(B), AF467074; *tet*(D), AP010961; *tet*(K), AJ888003; *tet*(M), X90939; *tet*(Q), Z21523; *tet*(X), AB097942). For MIC assays, ampicillin was present at a constant concentration of 50 μg/mL in each well to maintain plasmids; cells were grown in cation-adjusted Mueller Hinton Broth (MHB; BBL/ BD, Franklin Lakes, NJ) containing ampicillin, 50 μg/mL, and pre-induced for 30 minutes with 1% arabinose (*tet*(A), *tet*(B), *tet*(D), *tet*(M), *tet*(Q), *tet*(X)) or 0.1% arabinose (*tet*(K)) at 25°C prior to use as inocula in MIC assays.

Materials. The 4,7,8-trisubstituted tetracycline analogs **3** were prepared from an *N,N*-diallylamino enone and D-ring precursors via a Michael-Dieckmann annulation, followed by de-allylation, derivatization, and de-protection (Scheme 1).

Results

Scheme 1. Representative Synthesis of 4,7,8-trisubstituted Tetracycline Analogs

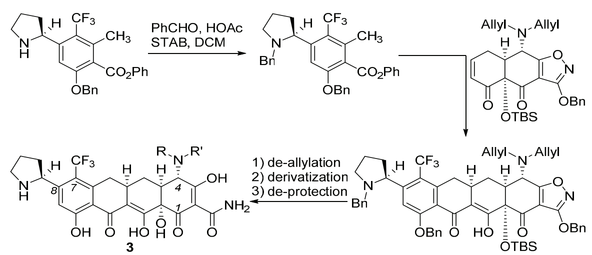
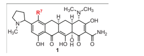


Table 1. *In Vitro* Activity of C7-substituted C8-pyrrolidinyl Tetracycline Analogs



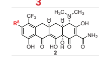
Cpd[a]	R^7	MIC (μg/mL)[b]																
		SA101	SA161	SA158	EF327	EF404	SP160	EC107	EC155	EC878	KP457	PM385	PA555	PA556	ECl603	AB250	SM256	BC240
		29213	*tet* (M)	*tet* (K)	*tet* (M)	*tet* (M)	*tet* (M)	25922	*tet* (A)	tolC	CTX-M-15		BAA-47		*tet* (A)			
1a	H	0.5	32	0.06	16	8	1	0.12	1	0.5	1	1	16	0.06	2	2	1	8
1b	F	0.25	16	0.03	8	2	0.25	0.06	1	0.12	1	2	8	0.12	2	1	0.5	8
1c[c]	Cl	0.12	1	≤0.016	1[d]	nd	0.5	≤0.016	1	n/t[e]	n/t	n/t	n/t	n/t	n/t	0.5	0.12	8
1d	CH_3	0.12	8	0.03	4	2	0.12	0.03	0.5	≤0.016	0.25	1	16	0.06	1	1	0.25	16
1e[c]	CN	1	32	0.25	16	16	1	0.5	16	0.12	8	8	>32	1	32	4	16	16
1f	CHF_2	16	>32	8	>32	>32	>32	8	32	8	>32	>32	>32	8	>32	>32	32	>32
1g	**CF_3**	**0.06**	**1**	**≤0.016**	**1**	**0.25**	**≤0.016**	**≤0.016**	**0.5**	**≤0.016**	**1**	**2**	**8**	**1**	**4**	**0.12**	**0.06**	**4**
1h[c]	NH_2	32	>32	16	>32	>32	32	32	>32	16	>32	>32	>32	16	>32	>32	>32	>32
1i[c]	OH	32	>32	16	>32	>32	>32	16	>32	16	>32	>32	>32	8	>32	>32	>32	>32
1j[c]	$N(CH_3)_2$	0.5	>32	0.12	32[d]	n/t	2	0.12	2	n/t	n/t	n/t	n/t	n/t	n/t	16	2	>32
1k[c]	OCH_3	0.25	8	0.06	8[d]	n/t	0.5	0.06	1	n/t	n/t	n/t	n/t	n/t	n/t	4	2	16
1l	OCF_3	0.12	4	≤0.016	4	1	≤0.016	≤0.016	0.25	≤0.016	1	2	8	0.5	2	0.12	0.12	4
Tigecycline		0.12	0.5	0.06	0.03	0.03	≤0.016	0.03	1	0.06	1	2	16	0.12	2	4	0.5	8

[a]Single diastereomer unless otherwise noted. [b]SA: *Staphylococcus aureus*; EF: *Enterococcus faecalis*; SP. *Streptococcus pneumoniae*; EC: *Escherichia coli*; KP. *Klebsiella pneumoniae*; PM: *Proteus mirabilis*; PA: *Pseudomonas aeruginosa*; ECl: *Enterobactor cloacae*; AB: *Acinetobacter baumannii*; SM: *Stenotrophomonas maltophilia*; BC: *Burkholderia cepacia*. [c]A mixture of two diastereomers (~1:1). [d]Screened against a different isolates of *E. faecalis* (EF159) with similar genetic and drug susceptibility profiles for EF327, also expressing the *tet*(M) resistance gene. [e]n/t = not tested.

- Compound **1g**, with a CF_3 group at C7, has the most overall potency in this sub-series

Results (cont'd)

Table 2. *In Vitro* Activity of C7-CF_3 C8-heterocyclyl Tetracycline Analogs

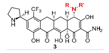


Cpd[a]	R^8	MIC (μg/mL)																
		SA101	SA161	SA158	EF327	EF404	SP160	EC107	EC155	EC878	KP457	PM385	PA555	PA556	ECl603	AB250	SM256	BC240
		29213	*tet* (M)	*tet* (K)	*tet* (M)	*tet* (M)	*tet* (M)	25922	*tet* (A)	tolC	CTX-M-15		BAA-47		*tet* (A)			
2a		0.25	32	0.12	8	4	0.5	≤0.016	4	0.03	0.25	1	4	0.03	8	4	2	8
2b		0.12	4	≤0.016	4	2	0.12	≤0.016	1	≤0.016	2	2	8	0.25	4	0.0625	0.0625	4
2c		**0.12**	**2**	**≤0.016**	**1**	**0.12**	**≤0.016**	**≤0.016**	**2**	**≤0.016**	**0.12**	**0.25**	**2**	**≤0.016**	**8**	**1**	**0.5**	**4**
1g		0.0625	1	≤0.016	1	0.25	≤0.016	≤0.016	0.5	≤0.016	1	2	8	1	4	0.12	0.0625	4
2d		0.03	1	≤0.016	0.5	0.12	≤0.016	≤0.016	0.5	0.03	4	4	32	2	4	1	1	8
2e		0.12	4	≤0.016	0.5	0.12	≤0.016	≤0.016	2	≤0.016	0.5	1	16	0.0625	8	1	1	4
2f		0.12	1	≤0.016	0.12	≤0.016	≤0.016	≤0.016	0.25	≤0.016	2	2	16	0.5	4	0.25	0.12	4
2g		0.12	8	≤0.016	16	4	0.5	0.12	32	≤0.016	4	4	32	0.12	>32	1	1	4
2h		0.25	4	≤0.016	>32	2	0.12	0.5	32	0.5	32	8	>32	4	>32	2	4	16
2i		0.5	32	0.25	4	4	1	0.25	4	0.5	2	8	32	0.5	8	>32	16	>32
2j		1	16	0.12	8	2	0.25	0.5	4	0.5	4	16	32	1	16	>32	16	>32
2k		0.25	2	≤0.016	2	0.5	0.03	0.12	1	0.03	2	8	32	0.25	4	2	1	16
2l		1	16	0.12	16	16	0.5	4	16	1	>32	>32	>32	4	>32	32	16	>32
2n[b]		2	8	0.25	8	8	0.5	8	16	2	>32	>32	>32	8	>32	32	>32	>32

[a]Single diastereomer unless otherwise noted. [b]A mixture of two diastereomers (~1:1).

- Compound **2c**, with a CF_3 group at C7 and an unsubstituted pyrrolidine at the C8 position, is the most potent analog in this sub-series, especially against the Gram-negative pathogens in the panel

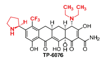
Table 3. *In Vitro* Activity of C4-substituted C7-CF_3 C8-pyrrolidinyl Tetracycline Analogs



Cpd	NRR'	MIC (μg/mL)																
		SA101	SA161	SA158	EF327	EF404	SP160	EC107	EC155	EC878	KP457	PM385	PA555	PA556	ECl603	AB250	SM256	BC240
		29213	*tet* (M)	*tet* (K)	*tet* (M)	*tet* (M)	*tet* (M)	25922	*tet* (A)	tolC	CTX-M-15		BAA-47		*tet* (A)			
3a	NH_2	4	>32	0.5	32	16	4	1	>32	0.25	4	2	32	0.12	>32	>32	16	>32
3b	$NHCH_2CH_3$	1	>32	0.25	16	4	1	0.12	32	0.06	0.5	1	8	0.06	32	8	8	32
3c	$NHCH_2CH_2CH_3$	1	32	0.5	8	2	0.25	≤0.016	2	≤0.016	0.25	0.25	8	0.03	2	4	2	4
3d	NH^iPr	1	32	0.25	8	2	0.25	≤0.016	2	0.03	0.5	0.5	8	0.12	8	4	2	8
3e	$NH(CH_2)_3OH$	>32	>32	16	>32	>32	32	16	>32	2	32	>32	>32	2	>32	>32	>32	>32
2c	$N(CH_3)_2$	0.06	4	≤0.016	1	0.25	≤0.016	≤0.016	2	≤0.016	0.12	0.25	2	0.03	8	2	1	4
3f		4	>32	0.5	32	4	0.25	0.06	16	≤0.016	0.5	2	16	0.06	16	16	32	32
3g	$N(CH_3)CH_2CH_3$	0.06	2	≤0.016	0.5	0.12	≤0.016	≤0.016	0.25	≤0.016	0.03	0.25	8	0.03	2	0.25	0.12	2
3h		0.5	1	0.12	1	0.25	0.25	0.06	1	0.12	2	4	>32	2	8	2	0.5	>32
3i		1	32	0.25	8	2	0.25	≤0.016	4	≤0.016	0.25	1	16	0.06	16	8	4	8
3j	$N(CH_3)Pr$	0.03	0.5	≤0.016	0.5	0.06	≤0.016	≤0.016	0.12	≤0.016	0.25	1	16	0.12	2	0.5	0.12	4
3k	$N(CH_3)^iPr$	0.25	8	0.06	2	0.5	0.06	≤0.016	1	≤0.016	0.25	1	>32	0.25	4	1	0.5	4
TP-6076	**$N(CH_2CH_3)_2$**	**0.03**	**0.5**	**0.03**	**0.12**	**0.03**	**≤0.016**	**≤0.016**	**≤0.016**	**≤0.016**	**0.03**	**0.12**	**4**	**0.03**	**0.5**	**0.12**	**0.06**	**1**
3l(B)	$N(CH_2CH_3)_2$	0.25	16	0.06	8	2	0.5	≤0.016	0.25	≤0.016	0.25	1	16	0.25	1	0.25	0.25	4
3m		1	2	0.25	1	0.5	0.5	0.12	1	0.25	2	4	>32	4	16	4	1	>32
3n	$N(CH_2CH_3)Pr$	0.25	4	0.06	1	0.25	≤0.016	≤0.016	0.03	≤0.016	0.25	1	16	1	2	0.25	0.12	4
3o	$N(CH_2CH_3)^iPr$	0.5	4	0.06	0.5	0.12	≤0.016	≤0.016	0.06	≤0.016	0.25	1	8	0.5	1	0.25	0.25	4
3p	NPr_2	2	2	2	2	2	1	0.12	0.5	0.5	2	4	16	8	8	4	2	>32

- Compound TP-6076, a 4-diethylamino-7-trifluoromethyl-8-pyrrolidinyltetracycline analog, is the most potent compound overall against Gram-negative and Gram-positive pathogens in the panel

Table 4. MIC_{90} Values of TP-6076 against Gram-negative Pathogens



MIC90 Values of TP-6076: Gram-negatives

Antibiotic	MIC_{90} (μg/mL)										
	E. coli (n = 253)	*E. coli* ESBL (n = 101)	*E. cloacae* (n = 36)	*K. pneumoniae* (n = 235)	*K. pneumoniae* ESBL (n = 143)	*K. pneumoniae* CRE (n = 51)	*A. baumannii* (n = 62)	*A. baumannii 3rd gen ceph-R* (n = 62)	*A. baumannii* CRAB (n = 35)	*P. mirabilis* (n = 51)	*P. aeruginosa* (n = 75)
TP-6076	**0.03**	**0.03**	**0.25**	**0.5**	**0.5**	**0.25**	**0.06**	**0.06**	**0.06**	**0.5**	**16**
Tetracycline	>32	>32	16	>32	>32	>32	>4[a]	>4[a]	>4[a]	>32	>32
Tigecycline	0.5	0.5	4	4	4	2	4	4	4	8	32
Carbapenem[b]	0.06	0.25	1	32	>32	>32	>32	>32	>32	0.5	32
3rd Gen Ceph[c]	>32	>32	>32	>32	>32	>32	>64	>64	>64	0.25	>32
Fluoroquinolone[d]	32	32	8	>32	>32	>32	>32	>32	>32	32	>32
Aminoglycoside[e]	>32	>32	2	>32	>32	>32	>32	>32	>32	32	>32

[a]minocycline; [b]imipenem, ertapenem, or meropenem; [c]cefotaxime, ceftazidime, or ceftriaxone; [d]levofloxacin or ciprofloxacin; [e]gentamicin, amikacin, or tobramycin.

Table 5. MIC_{90} Values of TP-6076 against Gram-positive Pathogens

Antibiotic	MIC_{90} (μg/mL)				
	MRSA (n = 23)	*S. pneumoniae* (n = 24)	*S. pyogenes* (n = 21)	*E. faecalis* (n = 22)	*E. faecium* (n = 15)
TP-6076	**2**	**<0.016**	**0.12**	**0.5**	**0.5**
Tetracycline	>32	>32	32	>32	>32
Tigecycline	0.5	0.03	0.03	0.06	0.06
Meropenem	>32	1	<0.016	16	>32
Fluoroquinolone	64	8[a]	1	>32	>32
Aminoglycoside	>32	16	8	>32	>32

[a]23 isolates

Table 6. TP-6076 MIC against *E. coli* DH10B Recombinantly Expressing Tetracycline Resistance Genes

Antibiotic	MIC (μg/mL)								
	EC107	EC969	EC2152	EC970	EC1082	EC1083	EC1827	EC1153	EC971
	25922	*tet* (M)	*tet* (Q)	*tet* (K)	*tet* (A)	*tet* (B)	*tet* (D)	*tet* (X)	lacZ
TP-6076	**0.008**	**0.03**	**0.03**	**0.004**	**0.06**	**0.03**	**0.016**	**0.25**	**0.008**
Tetracycline	1	>64	64	>64	>64	>64	>64	>64	2
Minocycline	0.25	>32	>32	1	8	4	8	2	0.5
Doxycycline	1	32	32	8	32	32	32	8	1
Tigecycline	0.03	0.25	0.12	0.12	1	0.5	0.25	2	0.12
Ceftriaxone	0.06	0.12	0.12	0.06	0.06	0.06	0.06	0.12	0.12

Table 7. TP-6076 MIC against *K. pneumoniae* with *ramA* Mutations

Antibiotic	MIC (μg/mL)				
	ramA on Chromosome			*ramA* on Plasmid	
	KP987	KP986	KP989	KP988	KP990
	wt *ramA*	Upregulated *ramA*	KP986 with Deleted *ramA*	KP987 with Overexperssed *ramA*	KP989 with Overexperssed *ramA*
TP-6076	**0.03**	**1**	**0.016**	**2**	**2**
Tetracycline	4	32	4	32	>32
Tigecycline	0.5	4	0.25	16	16
Levofloxacin	0.06	1	0.06	1	2

Table 8. TP-6076 MIC against Mutant *A. baumannii* with Altered *adeABC* Efflux Expression

Antibiotic	MIC (μg/mL)				
	AB991	AB992	AB993	AB994	AB995
	G4904 wt parent of G5139	G5141 wt parent of G5140 & GC7951	G5140 overexpressing *adeAB*	G5139 overexpressing *adeAB*	GC7951 knockout of *adeB*
TP-6076	**0.008**	**0.008**	**0.12**	**0.12**	**0.016**
Tetracycline	32	32	32	32	>32
Tigecycline	1	1	8	8	2
Meropenem	2	2	2	2	2
Levofloxacin	16	>32	32	32	16
Gentamicin	>32	>32	>32	>32	32

Table 9. MIC_{50}/MIC_{90} Values of TP-6076 against Carbapenem Resistant Organisms

Antibiotic	*Enterobacteriaceae* (n = 178)			*A. baumannii* (n = 80)			*P. aeruginosa* (n = 76)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
TP-6076	**0.25**	**1**	**0.03-8**	**0.06**	**0.12**	**≤0.008-1**	**8**	**16**	**1->16**
Tigecycline	4	8	0.5->16	4	8	0.5-16	>16	>16	4->16
Ceftazidime	>16	>16	1->16	>16	>16	8->16	>16	>16	4->16
Imipenem	>16	>16	4->16	>16	>16	16->16	>16	>16	8->16
Ceftolozane/Tazobactam	>16	>16	>2->16	>16	>16	8->16	>16	>16	2->16
Ceftazidime/Avibactam	4	>16	0.25->16	>16	>16	16->16	>16	>16	4->16

Conclusions

- Using the Tetraphase total synthesis platform, a wide range of tetracycline analogs with novel substitutions at C4, C7, and C8 were systematically explored.
- Among the tetracycline analogs investigated, the C7-CF_3 and OCF_3 groups are preferred for optimal antibacterial activity. For the C8 substitutions, compound **2c** with a pyrrolidine had the most overall activity against Gram-negative pathogens.
- Further exploring the C4 position, the diethylamino analog TP-6076 was discovered to have the best activity across the entire primary panel.
- TP-6076 consistently showed 2- to 64-fold greater potency by MIC_{90} than tigecycline against MDR Gram-negative pathogens, including carbapenem-resistant *Enterobacteriaceae* and *A. baumannii*, and had comparable or better MIC_{90} values against all other clinical comparators tested.
- TP-6076 retained activity *in vitro* against Gram-negative pathogens, including those expressing tetracycline-specific and intrinsic resistance mechanisms affecting the existing tetracycline-class of antibiotics.
- The *in vitro* potent antibacterial activity of TP-6076 also translated to high *in vivo* efficacy in several mouse infection models challenged with MDR Gram-negative isolates.[vi] TP-6076 is currently being evaluated in phase 1 clinical studies.

References

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