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New Antibacterial Tetrahydro-4(2*H*)-thiopyran and Thiomorpholine *S*-Oxide and *S*,*S*-Dioxide Phenyloxazolidinones

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Abstract—Combinatorial libraries of *N*-acylated 5-(*S*)-aminomethyloxazolidinone derivatives of *S*-oxide and *S*,*S*-dioxide tetrahydro-4(2H)-thiopyranyl and thiomorpholine phenyloxazolidinone series have been synthesized on a solid phase and evaluated for antimicrobial activity. Several novel potent leads have been identified, including orally active oxazolidinones with enhanced activity against respiratory tract infection pathogens *Haemophilus influenzae* and *Moraxella catarrhalis*.
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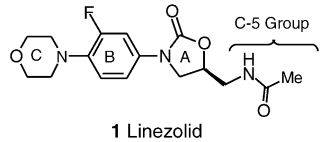
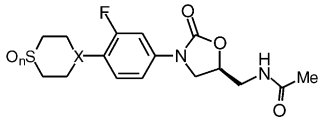
Rapid proliferation of drug-resistant bacteria is a serious problem in hospitals and community.[1–3] Recent emergence of glycopeptide-resistant *Staphylococcus aureus* strains with reduced susceptibility to vancomycin underscores an urgent need to discover and develop new antimicrobial agents that act via novel mechanisms.[4,5] Oxazolidinones are a new class of orally active synthetic antibacterial agents that work by inhibiting protein synthesis at ribosomal level.[6–8] Their unique mode of action offers a potential for low cross-resistance with existing antimicrobial protein synthesis inhibitors.[9]

Clinical success of the first oxazolidinone drug linezolid **1** (Zyvox®) has led to increased efforts toward second generation oxazolidinone antiinfectives with an improved potency and spectrum.[10] Most of the current research has been focused around new replacements for the morpholine C-ring featured in linezolid **1**. Thus, four new promising C-rings, tetrahydro-4-(2*H*)-thiopyran sulfone or sulfoxide and thiomorpholine *S*-oxide or *S*,*S*-dioxide have been discovered by Pharmacia scientists.[11] Leads **2**–**5** have attractive in vitro and in vivo potency against gram-positive organisms and interesting activity versus fastidious gram-negative bacteria.[12] In contrast to C-ring modifications, 5-acetamidomethyl group variations have received relatively little attention in oxazolidinone research.[13–16] We have surveyed C-5 group analogues of tetrahydro-4(2*H*)-thiopyran and thiomorpholine oxazolidinones to explore SAR and discover new potent leads. Herein, we report the synthesis and evaluation of novel C-5 group variants in thiopyran and thiomorpholine series.



1 Linezolid



2 n = 1, X =N; **3** n = 2, X = N
4 n = 1, X = CH ; **5** n = 2, X = CH

Combinatorial solid-phase synthesis has been selected as a method of choice for an expedient study of novel C-5 group analogues. Four chemical classes of C-5 group

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analogues were targeted: amides, ureas, thioureas and carbamates. The choice of C-5 group variants was made with attention to the groups outside of the scope of an early SAR. Structures were selected to encompass a broad range of physicochemical parameters: lipophilic, hydrophilic, acidic, basic, and so on. These also included amide and urea derivatives with a potential for base pair interactions with the bacterial RNA target. In this study, two complementary solid phase syntheses have been employed (Scheme 1).[17] Thus, pharmacophoric amines **6** and **7** were immobilized on Sasrin-derived dialkoxybenzyl chloride, whereas amines **8** (a *trans* sulfoxide configuration) and **9** were immobilized on BAL-linker aldehyde resin via reductive alkylation. In the latter protocol, an excess of unreacted amine was recovered upon imine formation (prior to a sodium cyanoborohydride reduction step). The imine formation was quantified by spectrophotometry upon releasing the amine from the resin sample via hydrazine treatment. The resulting resin-bound secondary amines have been converted to amides using standard coupling conditions (Scheme 1). Alternatively, the immobilized amine was treated with a diverse set of isocyanate, isothiocyanate and carbamoyl chloride reagents to generate urea, thiourea, and carbamate products, respectively. The resulting amine derivatives were released from solid support via TFA cleavage. A total of 379 discrete compounds were synthesized as a set of four libraries.[18]

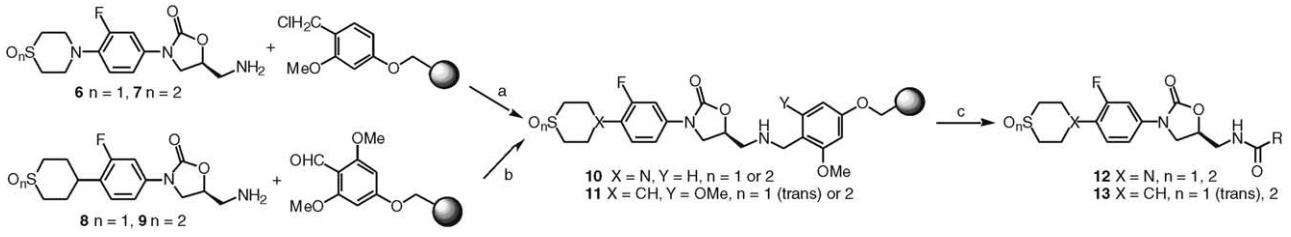
In Vitro Antimicrobial Evaluation

Libraries have been screened against a panel of gram-positive (*S. aureus* strain UC9213, linezolid-resistant *S. aureus* (Pharmacia in vitro isolate of *S. aureus* ATCC29213 selected for linezolid resistance, with G2447U and G2576U 23S rRNA mutations (*Escherichia coli* numbering), *Staphylococcus epidermidis* strain 30031, *Streptococcus pneumoniae* strain ATCC6305 and vancomycin-resistant *Enterococcus faecium* strain UC12712 and fastidious gram-negative (*Haemophilus influenzae* strain 30063 and *Moraxella catarrhalis* 30607) bacteria in a high-throughput whole cell assay.[19] A total of 26 hits with activity comparable to that of linezolid were identified. These hits were resynthesized and purified using conventional solution-phase chemistry. Selected analogues were evaluated against an expanded panel of microorganisms following NCCLS standards (total of 14 bacterial strains; see Table 1 for selected minimum inhibitory concentration data).[20]

The following SAR trends have been observed. Conservative deviations from the classical 5-(*S*)-acetamido group in both the sulfoxide and sulfone thiomorpholine series result in a loss of the antibacterial potency as exemplified by isoamyl-substituted derivative **12b**, O-Me glycolamide **12j**, and trifluoroethyl analogue **12o** (*S. aureus* MICs $\geq$64, 16–32, and 8–16 μg/mL, respectively). A similar trend is observed with aromatic C-5 groups. In particular, incorporation of *ortho*-substituted aroyl structures appears detrimental for activity. Thus, the sterically hindered 2,5-dimethoxyphenyl analogue **12a** is 2- to 4-fold less active than the benzamide **12d**, possibly reflecting a requirement for a planar arrangement between the amide functionality and a terminal substitutent (e.g., *S. pneumoniae* MICs 8–16 and 32 μg/mL for the two compounds, respectively). Certain heteroaroyl C-5 groups are permitted. Thus, the 1,2,3-thiadiazole analogue **12c** is ca. 2-fold more potent than benzamide **12d** (*S. pneumoniae* MICs 4 and 8–16 μg/mL, respectively).

As an exception, cinnamamide C-5 groups are well tolerated in terms of gram-positive activity, as exemplified by the potency of analogues **12e**–**12i** (MIC range of 1–8 and 0.5–2 μg/mL for *S. aureus* and *S. pneumoniae* strains, respectively). The potency of these derivatives is suggestive of an additional binding interaction(s) afforded by the extended C-5 structure (such as complementary π-stacking interactions at the 23S rRNA target). This structural variation is accompanied by overall attenuation of the gram-negative potency, likely due to a reduced outer membrane permeability and/or efflux. Within the C-5 cinnamamide series, incorporation of electron-withdrawing *para*-substitutents is beneficial. Thus, the chlorophenyl derivative **12f** and methoxime **12h** are ca. 2–4-fold more active than methoxyphenyl analogue **12g** and para-acetamide **12i**. This observation underscores a potential for C-ring-specific effects resulting from C-5 group variations. C-5 cinnamamides **12f** and **12h** as well as difluoroacetamide **13f** compare favorably to linezolid in gram-positive assays: *S. aureus* and *S. pneumoniae* MICs 1–4 and 0.5–1 μg/mL for the two microorganisms, respectively.

Thiomorpholine *S*,*S*-dioxide C-ring analogues are somewhat more potent than the respective *S*-oxide derivatives (cf., e.g., compounds **12c** and **12n**, **12k** and **12p**). Within the thiomorpholine series, none of the new C-5 analogues appear significantly superior to acetamide derivative **3**.



Scheme 1. Solid-phase library synthesis: (a) DMF; (b) $NaBH_3CN$, MeOH, DMF, 1% AcOH; (c) (1) RCOOH, HATU, DIEA, DCM, rt or RNCO, DMF or RNCS, DMF or ROCOCl, DIEA, DMF; (2) TFA, DCM.

Table 1. MICs (μg/mL) for selected thiomorpholine **12** and tetrahydro-4(2*H*)-thiopyran **13** phenyloxazolidinone *S*-oxide and *S*,*S*-dioxides

Entry	R	X	*n*	SA (4)	LRSA (1)	SE (3)	VRE (1)	SPN (2)	HI (2)	MC (2)
1	Linezolid		—	2–4	64	1–2	4	0.5–1	8–16	4–8
2	$-CH_3$	N	1	4–8	>64	1–2	2	1	4–8	4
12a	$2,5\text{-}(CH_3O)_2C_6H_3$	N	1	≥64	>64	32–64	>64	32	>64	>64
12b	$-CH_2CH_2CH(CH_3)_2$	N	1	≥64	>64	32–>64	>64	32	>64	>64
12c	−1,2,3-Thiadiazole-4-yl	N	1	16–32	>64	8	16	4	=64	32–>64
12d	$-C_6H_5$	N	1	32–64	>64	16–32	32	8–16	>64	>64
12e	$CH{=}CH(4\text{-}FC_6H_4)$	N	1	4–8	32	2	4	1	32–64	16–32
12f	$CH{=}CH(4\text{-}ClC_6H_4)$	N	1	2–4	8	1	2	0.5	32–>64	8
12g	$CH{=}CH(4\text{-}CH_3OC_6H_4)$	N	1	4	>64	2	4	1	>64	>64
12h	$CH{=}CH(4\text{-}CH_3ON{=}CHC_6H_4)$	N	1	1–2	64	0.5–1	2	0.5–1	>64	4
12i	$CH{=}CH(4\text{-}AcNHC_6H_4)$	N	1	4–8	>64	1–2	8	2	>64	16
12j	$-CH_2OCH_3$	N	1	16–32	>64	4–8	16	4–8	32–>64	16–32
12k	–Cyclopropyl	N	1	16–32	64	4	4	2	32	8–16
12l	$-CF_3$	N	1	>64	>64	>64	>64	>64	>64	>64
3	$-CH_3$	N	2	4	64	1	2	0.5–1	4–8	4
12m	$-CF_3$	N	2	16–32	16	8	16	4	≥64	32–>64
12n	−1,2,3-Thiadiazole-4-yl	N	2	8–32	32	4–8	16	4–8	32–64	32
12o	$-CH_2CF_3$	N	2	8–16	>64	4	4	2	16–32	16
12p	–Cyclopropyl	N	2	4–16		2	4	2	16–32	4
4	$-CH_3$	CH	1	4	64	0.5–1	2	0.5	8	8–16
13a	$-C_6H_4OCOCH_3$	CH	1	8–32	64	4–8	0.06	4	=64	32–64
13b	$CH{=}CH(4\text{-}AcOC_6H_4)$	CH	1	2–8	16	1–2	8	1	32	8–16
13c	$CH{=}CH(4\text{-}HOC_6H_4)$	CH	1	2–8	>64	1–2	4	1	>64	8–16
13d	$-CH_2OH$	CH	1	16–32	64	4	8	2–4	8	8
13e	$-CH_2F$	CH	1	2–4	32	1–2	4	0.5–1	4–8	8
13f	$-CHF_2$	CH	1	2–4	16	1–2	2	0.5	4–8	4–8
13g	$-CF_3$	CH	1	8	32	2–4	4	2	8–32	8
5	$-CH_3$	CH	2	2–4	32	0.5	2	0.5	4–8	2–4
13h	$-C_6H_3Cl_2$	CH	2	>64	>64	2–4	>64	4–8	>64	>64
13i	$CH{=}CH(3\text{-Py})$	CH	2	4–16	32	2–4	8	2	16–64	16–32
13j	$-CH(OH)CH_2CH_2NH_2$	CH	2	>64	>64	>64	32–64	>64	>64	>64
13k	$-CH_2SCH_3$	CH	2	4–8	64	2	4	2	8–32	8
13l	$-CH_2F$	CH	2	2	64	0.5–1	2	0.5	4–16	8
13m	$-CF_3$	CH	2	8–32	64	2–4	8	2	8–32	8
13n	$-CHF_2$	CH	2	4–16	32	1–2	4	1	4–16	8
13o	$-NH_2$	CH	2	16	>64	0.13–2	4	1–2	4–16	8–16

Strains (number of strains in parentheses): SA, *Staphylococcus aureus* strains UC12673, UC9213, ATCC29213, and UC9271; LRSA, Pharmacia in vitro isolate of *S. aureus* ATCC29213 selected for linezolid resistance (has G2447U and G2576U (*Escherichia coli* numbering) 23S rRNA mutations); SE, *S. epidermidis* strains UC12084, 30031, and CDC-CNS90; SPN, *S. pneumoniae* strains ATCC6305 and 31573; VRE, vancomycin-resistant *E. faecium* UC12712; HI, *H. influenzae* strains 30063 and ATCC31517; MC, *M. catarrhalis* strains 30607 and 30603.

Table 2. Single-dose pharmacokinetics of **13f** in male Sprague–Dawley rat

Route	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}z$ (h)	AUC (0, ∞) (mg X h/mL)	V_{ss} (L/kg)	CL (mL/min/kg)	*F* (%)
IV	9.13±0.85	15.6±1.5	0.033±0.00	2.9±1.9	4.09±0.48	1.78±0.77	37.25±0.93	*n*
PO	19.01±0.31	3.47±0.14	0.38±0.18	3.6±3.3	6.57±0.43	*n*	—	77.1±4.3
PO	76.4±2.6	8.6±2.1	0.50±0.00	4.25±0.59	32.4±1.5	29.84±0.54	—	95.1±8.0
PO	158.5±2.0	9.07±2.0	0.38±0.18	7.93±0.23	60.8±2.1	29.84±0.54	—	85.7±4.6

In vitro antimicrobial data for both the sulfoxide and sulfone analogues of tetrahydro-4(2*H*)-thiopyran phenyloxazolidinones suggest that C-5 amides are more active than ureas, thioureas and carbamates. Simple substitution of the C-5 acetamide with either fluorine or sulfur leads to 2-fold better potency against *H. influenzae* (cf. also *M. catarrhalis*) as exemplified by compounds **13e**, **13f**, **13k**, and **13l**. Large C-5 groups such as aromatic and heteroaromatic rings as well as bulky alkyl groups are not tolerated in either sulfoxide or sulfone series, as exemplified by compounds acetylated with 4′-acetoxyphenyl **13b**, 3,4-dichlorophenyl **13h** and α-hydroxyaminopropyl **13j**. As in the thiomorpholine series, cinnamoyl analogues **13b** and **13c** have good gram-positive antimicrobial activity (*S. aureus* MICs 2–8 μg/mL) with a notable tolerance toward permutations in the aromatic ring of a C-5 substitutent. Polar or basic C-5 groups are not tolerated: see (e.g., inactive compounds **13d** and **13j**). Notably, new leads **13e** and **13f** are ca. 2-fold more potent than linezolid against *H. influenzae* (cf. also *M. catarrhalis*). Fluoro analogues **13e** and **13f** are essentially equipotent to the parent C-5 acetamide leads **4** and **5**.

In Vivo Antimicrobial Evaluation

Several compounds have been evaluated in a *S. aureus* mouse septicemia model[12] (po administration). Compound **13e** (ED_{50} 3.75 mg/kg) displayed an oral efficacy

slightly superior to that for linezolid **1** (ED_{50} 5 mg/kg), whereas analogue **13f** (ED_{50} 6.52 mg/kg) was comparable to the drug.

Pharmacokinetic studies were performed on male Sprague–Dawley rats each fitted with a cannulas implanted in the superior vena cava via the jugular vein for iv dosing and blood sample collection. iv and po doses were administered to fasted rats in a parallel fashion (two animals per dose). Serial specimens (250 μL) were collected and quantitation performed using a standard APCI-LC/MS/MS method. The novel difluoro analogue **13f** displayed good oral bioavailability. In a po dose escalation study, a linear increase in drug exposure was observed as indicated by AUC. The compound was very rapidly absorbed at all doses with T_{max} occurring within 0.5 h (see Table 2).

Conclusions

An SAR study of C-5 amide analogues of *S*-oxide and *S*,*S*-dioxide thiomorpholine and thiopyran oxazolidinones was performed and several novel leads with good in vitro potency against gram-positive bacteria were identified. The SAR of this series indicates a preference for small-sized lipophilic C-5 groups with the exception of well tolerated extended cinnamamides. The new lead **13f** displayed excellent oral efficacy in mice and favorable pharmacokinetics in a rat model.

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18. Each library compound was characterized by HPLC and ESI-MS. In addition, ca. 15% of randomly selected library members were characterized by ^{1}H NMR and their yields were estimated using *t*BuOH as an internal standard. Analogues were prepared in four libraries: 226 amides, 20 ureas, 22 thiourea, 11 carbamate.
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