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Novel Oxazolidinone–Quinolone Hybrid Antimicrobials

Mikhail F. Gordeev,[a,*] Corinne Hackbarth,[a] Michael R. Barbachyn,[b] Lee S. Banitt,[b] James R. Gage,[b] Gary W. Luehr,[a] Marcela Gomez,[a] Joaquim Trias,[a] Sara E. Morin,[b] Gary E. Zurenko,[b] Christian N. Parker,[b] Jonathan M. Evans,[b] Richard J. White[a] and Dinesh V. Patel[a]

[a]*Vicuron Pharmaceuticals Inc., 34790 Ardentech Ct., Fremont, CA 94555, USA*
[b]*Pharmacia Co., 7000 Portage Rd., Kalamazoo, MI 49001, USA*

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Abstract—Antimicrobial compounds incorporating oxazolidinone and quinolone pharmacophore substructures have been synthesized and evaluated. Representative analogues **2**, **5**, and **6** display an improved potency versus linezolid against gram-positive and fastidious gram-negative pathogens. The compounds are also active against linezolid- and ciprofloxacin-resistant *Staphylococcus aureus* and *Enterococcus faecium* strains. The MOA for these new antimicrobials is consistent with a combination of protein synthesis and gyrase A/topoisomerase IV inhibition, with a structure-dependent degree of the contribution from each inhibitory mechanism.

Oxazolidinones are a new class of synthetic antibacterial agents with a unique mechanism of bacterial protein synthesis inhibition (for recent reviews, see refs 1–5). The first drug in this class, Zyvox® (linezolid), has been widely accepted as a valuable addition to the chemotherapeutic armamentarium for treatment of serious gram-positive infections.[6,7] First generation oxazolidinones as represented by linezolid are generally limited in their antimicrobial spectrum to gram-positive species.[1–7] An expanded spectrum and enhanced potency of newer second generation oxazolidinones with activity against gram-negative pathogens could expand the utility of this class beyond the hospital setting into the treatment of infections in the community.

En route to novel oxazolidinones with an expanded antibacterial spectrum and improved potency, we have analyzed various drug classes with activity against gram-negative microorganisms. The essentially gram-positive spectrum of oxazolidinones can be partially attributed to their limited ability to reach the cytoplasmic RNA target of gram-negative prokaryotes. In contrast, quinolones possess a well-recognized capacity to permeate through the cell membranes of gram-negative microorganisms and achieve sufficient intracellular target exposure. From an SAR viewpoint, antibacterial compounds of both classes feature a heterocyclic amine presented as a popular C-ring in oxazolidinones[1–3] and a mandatory cyclic amine in position 7 of quinolones.[8] We have envisaged a combination of substructures permitted by SAR for both oxazolidinone and quinolone antimicrobials (Fig. 1, structure I).[9,10] Given a precedence of N^1-aryl groups in quinolones (cf. tosufloxacin[8]), we have also hypothesized that the quinolone N^1 substituent may be supplanted by an oxazolidinone pharmacophore (e.g., structure II). Based on these considerations, we've set out to explore two distinct types of the hybrid classes I and II with potential for a dual MOA: protein synthesis inhibition (caused by the oxazolidinone) and the gyrase A/topoisomerase IV inhibition (due a to the quinolone pharmacophore). In a similar approach, quinolone–sulfonamide and quinolone–trimethoprim hybrids have been previously evaluated (see publications in refs 10 and 11 and refs cited therein). The quinolone structures have been also incorporated into 'codrug' cephalosporins designed to release the quinolone component upon β-lactam opening by bacterial enzymes.[12,13]

Synthesis of the prototypical analogues **2**, **5**, **6**, **13**, **16**, and **19** is summarized in Schemes 1–4. The compounds were tested against a panel of microorganisms including

*Corresponding author. Tel.: +1-510-739-3013; fax: +1-510-739-3050; e-mail: mgordeev@vicuron.com

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ciprofloxacin-resistant *Staphylococcus aureus* and *Enterococcus faecium*, and linezolid-resistant strains of *S. aureus* (LRSA) and *E. faecium* (LREF; Table 1). While the frequency of resistance to oxazolidinones is low, several clinical observations of resistance to linezolid have been reported.[14–18] It is expected that the incidence of resistance may rise over time, and compounds with LRSA and LREF coverage are of potential interest.

Design of a tether connecting the oxazolidinone and quinolone pharmacophores appears critical for antibacterial activity. Thus, N^1-linked tosufloxacin analogue **19** is inactive. In contrast, oxazolidinones **2**, **5**, and **6** incorporating ciprofloxacin, ofloxacin, and levofloxacin quinolone substructures linked via a piperazine group are highly potent against gram-positive strains: MICs versus linezolid-susceptible *S. aureus* and *E. faecium* are ≤ 1 μg/mL (see Table 1). Notably, the activity against gram-positive strains is markedly superior cf. to linezolid as well as versus progenitor eperezolid (the latter is equipotent to linezolid;[2] data omitted from Table 1). For example, compound **2** is ca. 8-fold more active than linezolid and eperezolid against both *S. aureus* and *E. faecium*. Analogues **2**, **5**, and **6** are also active against linezolid resistant strains, with MICs versus LRSA and LREF in the range of 2–4 and 0.5–4 μg/mL, respectively. It is also noteworthy that the compounds maintain

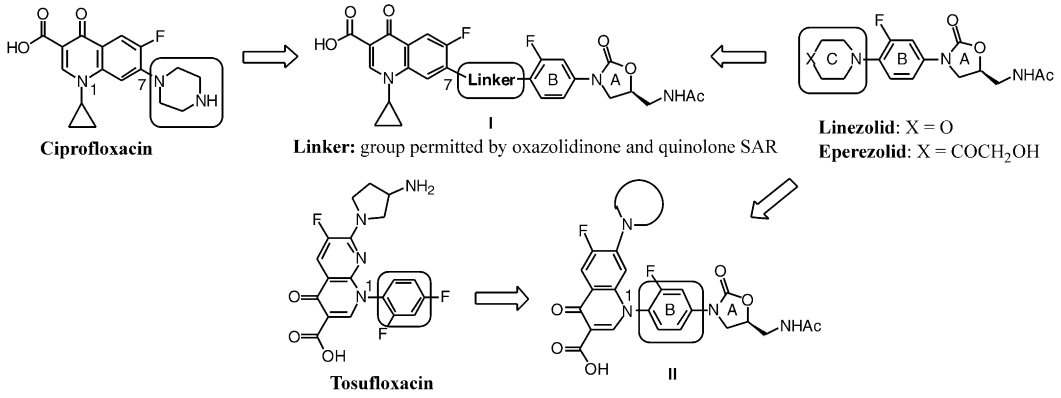
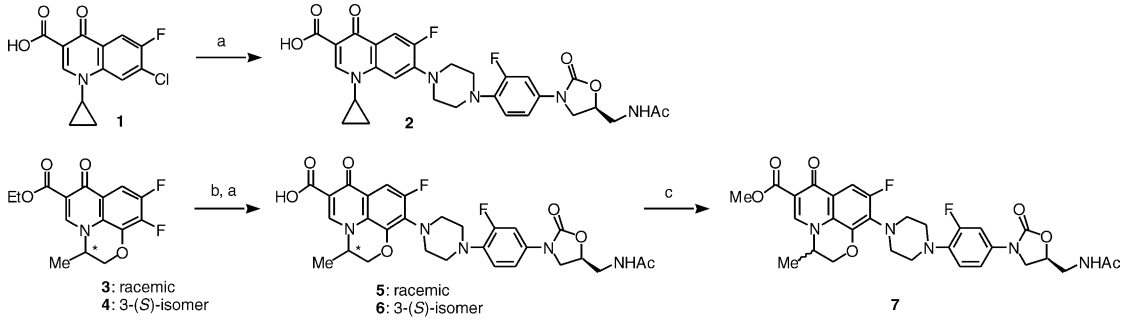
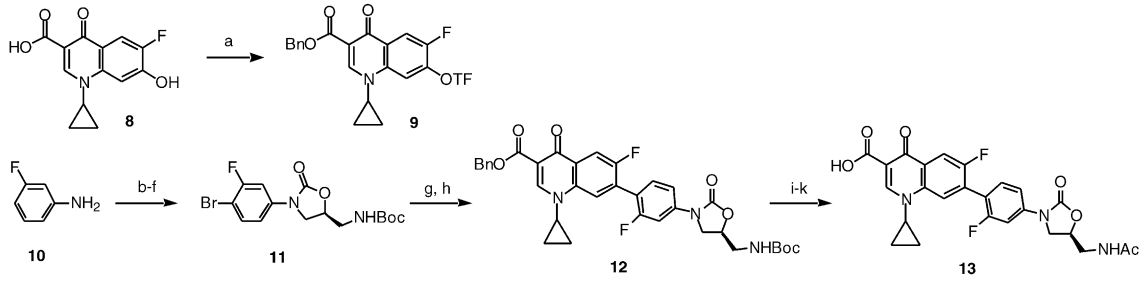


Figure 1. Conceptual design of oxazolidinone–quinolone antimicrobials.



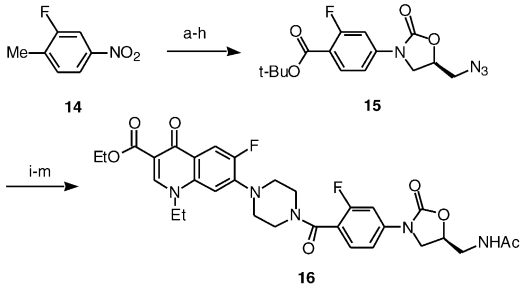
Scheme 1. Reagents and conditions: (a) 5-(*S*)-acetamidomethyl-3-[3-fluoro-(4-piperazine-1-yl)-phenyl]oxazolidinone, *N*-methylmorpholine, DMSO, 110 °C; (b) aq HCl–AcOH, D; (c) $TMSCHN_2$, MeOH, rt.



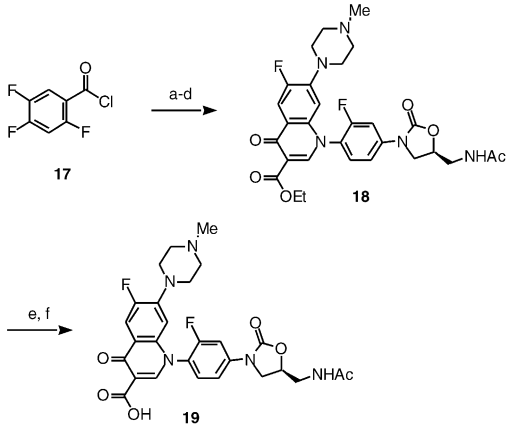
Scheme 2. Reagents and conditions: (a) Tf_2O, Py, 0 °C to rt; then BnOH, rt; (b) *i*-BuOCOCl, K_2CO_3, DCM, water, rt to Δ; (c) 1,3-dibromo-5,5-dimethylhydantoin, DCM, water, 39 °C; (d) *t*-$C_5H_{11}OLi$, THF, −15 °C; then pre-mixed at −25 °C to 10 °C (*S*)-3-chloro-1,2-propanediol, *t*-BuOK, THF; (e) 3-$NO_2C_6H_4SO_2Cl$, TEA, DCM; (f) 29% NH_4OH, MeCN, MeOH, sealed autoclave, 80 °C; (f) di-*t*-butylcarbonate; (g) EtMgBr, TMEDA, THF, −50 °C; then BuLi, hexanes, $B(OMe)_3$, then aq HCl; (h) **9**, $Pd(PPh_3)_2Cl_2$, aq K_2HPO_4, 1,2-dimethoxyethane, 90 °C; (i) TFA, DCM; (j) Ac_2O, Py; (k) 10% Pd/C, H_2, EtOH.

Table 1. Antimicrobial activity for oxazolidinone–quinolones (MICs, g/mL)[21]

Compd	SA1009 Cip^R	SA1011	SA1012 Lin^R	EF4010 Cip^R	EF4008 Lin^R; Cip^R	EF4016 Cip^R	EF4011 Lin^R; Cip^R	HI1008	MC1002	EC1008
Linezolid	4	4	64	4	16	2	8	16	8	>64
Ciprofloxacin	32	0.5	1	>64	>64	>64	>64	≤0.06	0.03	0.03
2	0.25	0.25	2	0.125	0.5	0.125	0.5	8	4	32
5	0.5	0.5	4	0.25	2	0.25	2	8	4	32
6	1	1	2	0.5	4	0.5	4	4	4	32
7	8	8	>64	8	>64	4	>64	>64	>64	>64
13	4	0.5	1	—	—	—	—	0.25	2	2
16	8	8	8	32	>64	64	>64	2	16	8
18	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
19	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64



Scheme 3. Reagents and conditions: (a) CrO_3, AcOH; (b) $SOCl_2$, DCM; (c) *t*-BuOLi, THF; (d) Fe, aq NH_4Cl; (e) CbzCl, Py, DCM; (f) (*R*)-glycidyl butyrate; (g) MsCl, TEA, DCM; (h) NaN_3, DMF, 80 °C; (i) PPh_3, THF, water, 55 °C; (j) Ac_2O, Py, DCM; (k) TFA, DCM, rt; (l) $PfpOCOCF_3$, Py, DMF; (m) norfloxacine, TEA, acetone.



Scheme 4. Reagents and conditions: (a) ethyl 3-dimethyl-aminoacrylate, TEA, dioxane, rt; (b) 5-(*S*)-acetamidomethyl-3-(3-fluoro-4-aminophenyl)oxazolidinone, 4-methylmorpholine, NMP, 110 °C; (c) DBU, NMP, 85 °C; (d) *N*-methylpiperazine, NMP, 80 °C; (e) aq HCl, 80 °C; (f) Ac_2O, polyvinylpyridine, MeCN.

activity against the quinolone-resistant *S. aureus* and *E. faecium* strains. For example, while ciprofloxacin has MIC of 32 μg/mL against the resistant *S. aureus* strain SA1009, MICs of hybrids **2**, **5**, and **6** are 0.25, 0.5 and 1 μg/mL, respectively.

Similar results were found for *E. faecium*. Thus, oxazolidinone-ciprofloxacin hybrids **2**, **5**, and **6** are ca. 32- to 128-fold more active than ciprofloxacin against quinolone-resistant *S. aureus* and *E. faecium* strains (Table 1). Furthermore, oxazolidinone-quinolone hybrids **2**, **5**, and **6** are 2–4 times more active than linezolid against two gram-negative respiratory tract pathogens, *Haemophilus influenzae* and *Moraxella catarrhalis*. However, these compounds were not as active vs. latter strains as the parent quinolones. The latter is likely reflective of a sub-optimal pK_a of the distal 7-amine group in the quinolone fragment of the compounds (due to a reduced basicity of *N*,*N*′-diarylpiperazine cf. to a mono-aryl piperazine linkage of progenitor quinolones; cf. also with only moderately active piperazine amide **16**).

The enhanced potency and expanded antibacterial spectrum of new analogues are consistent with anticipated dual oxazolidinone/quinolone MOA. In agreement with the quinolone SAR, the activity of ester **7** is inferior to that for corresponding acid **5**. As expected, 3-(*S*)-isomer **6** of the racemic (at quinolone) compound **5** appears somewhat more active than the latter against *H. influenzae*. This parallels SAR in the progenitor quinolone series, wherein 3-(*S*)-enantiomer levofloxacin is more potent than its racemic analogue, ofloxacin.[19,20] Attenuated activity of the biaryl hybrid **13** against ciprofloxacin-resistant *S. aureus* SA1009 is suggestive of an enhanced contribution of the quinolone MOA in this analogue vs. piperazine-linked compounds **2**, **5**, and **6**. Indeed, compound **13** exhibits an IC_{50} of 12.5 μM in a standard *Escherichia coli* DNA gyrase gel-based supercoil assay[22] (cf. to cipropfloxacin IC_{50} of 4.1 μM in the same assay). The hybrid **13** also displays atypical for oxazolidinones but expected for quinolones potency against a wild-type *E. coli* strain (MIC 2 μg/mL). This analogue is active against *H. influenzae*, *M. catarrhalis*, and LRSA: MICs 0.25, 2, and 1 μg/mL, respectively.

In summary, oxazolidinone and quinolone substructures merged in a mutually SAR-compatible design gave rise to a new class of antimicrobials with an improved spectrum and potency over linezolid. Prototypical analogues **2**, **5**, and **6** represent an improvement over linezolid against fastidious gram-negative pathogens *H. influenzae* and *M. catarrhalis*. These new leads also maintain activity against linezolid- and/or ciprofloxacin-resistant strains of *S. aureus* and *E. faecium*.

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21. MICs determined following NCCLS guidelines. (National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard*, 5th ed; NCCLS document M7-A5; NCCLS: Wayne, PA, 2000). Bacterial strains listed in Table 1 were obtained from Pharmacia Kalamazoo, MI, USA) and include: SA1009: clinical isolate of *S. aureus*; SA1011: ATCC29213; SA1012: in vitro selected linezolid-resistant strain derived from SA1011; EF4010 and EF4016: clinical isolates of *E. faecium*; EF4008 and EF4011: isogenic linezolid-resistant strains isolated from same patient as EF4010 and EF4016, respectively. HI1008: clinical isolate of *H. influenzae*; MC1002: clinical isolate of *M. catarrhalis*; EC1008: clinical isolate of *E. coli*.
22. A standard supercoiling assay using reagents and protocols obtained from TopoGen Inc., Columbus, OH, USA, was used to evaluate inhibitory activity of this compound.