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Novel 3-chlorooxazolidin-2-ones as antimicrobial agents

Timothy P. Shiau, Eric D. Turtle, Charles Francavilla, Nichole J. Alvarez, Meghan Zuck, Lisa Friedman, Donogh J. R. O'Mahony, Eddy Low, Mark B. Anderson, Ramin (Ron) Najafi, Rakesh K. Jain *

Novabay Pharmaceuticals, 5980 Horton St. Suite 550, Emeryville, CA 94608, United States

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ABSTRACT

Antimicrobial resistance against many known therapeutics is on the rise. We examined derivatives of 3-chlorooxazolidin-2-one **1a** (X = H) as antibacterial and antifungal agents. The key findings were that the activity and apparent in vitro cytotoxicity could be controlled by the substitution of charged solubilizers at the 4- and 5- positions. These changes both significantly increase the antifungal potency and decrease cytotoxicity. Particularly effective were trialkylammonium groups which led to 400- to 600-fold increases in the antifungal therapeutic index when compared to their unsubstituted counterparts.

Background and significance: Antimicrobial resistance is a rapidly evolving issue for which we felt could be addressed through the development of new organic chloroamines such as *N*-chlorooxazolidinone **1a**. While this compound has broad-spectrum antimicrobial activity it has generated limited interest beyond disinfecting water[1] or as an additive in cleaning agents.[2] Because chloroamines can rapidly react with a variety of targets which are essential to the invasive microbe, the potential for them to develop effective resistance to this oxidant is low. Our interest in these compounds as therapeutic agents for the treatment of topical infections such as conjunctivitis, otitis, wound care, impetigo, and skin and soft tissue infections (SSTI) have led us to more fully explore the structure–activity relationship (SAR) and structure-stability relationship (SSR) of *N*-chlorooxazolidinones.

Our mechanism of action experiments with *N,N*-dichloroamines (published separately) suggests that chloroamines act by oxidizing sulfur in methionines and cysteines, denaturing proteins. Based on our understanding, we anticipated *N*-chlorooxazolidinones to have N–Cl bonds that could be electronically controlled by the proximate functionalities, giving them more versatility, selectivity and other advantageous properties over simple inorganic chlorinating agents.[3] Moreover, they should exhibit efficacy and toxicity profiles which are chemically distinct from other therapeutic chloroamines such as *N*-chlorotaurine. Because the antimicrobial activity and oxidation potential is sensitive to the electronic and steric structure around the N–Cl bond, we systematically explored the antimicrobial activity by altering the electrophilic nature of the organic structure, using the heterocycle as a targeting moiety. We were also very interested in determining the role played by changing the hydrophilic or lipophilic nature of these groups on the properties and activities of the new heterocycles.

In this paper we describe a systematic chemical study of various 3-chlorooxazolidin-2-one derivatives to elucidate the effect of water-solubilizing and chemical structural features on the physical properties of the resulting analogs as well as their in vitro antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and fungi. These derivatives of 3-chloro-oxazolidin-2-one showed remarkable microbicidal activity against all strains tested while maintaining a suitable safety profile against mammalian cells.

Materials and methods: 4-Methyl-4-sulfonyl analogs were synthesized from the chloride **3**.[4] Displacement of the chloride with sodium sulfite, followed by N-chlorination of the oxazolidinone ring, gave sulfonate-solubilized **1b**. Similar displacement with ethanedithiol gave a mixture of disulfides **5a** ($n = 1$) and **5b** ($n = 2$). Oxidation of the mixture gave sulfone-extended sulfonate **6**, and N-chlorination yielded **1c**. Displacement of the alkyl chloride with potassium thioacetate gave thioacetate **7**; alkaline hydrolysis of the thioester, alkylation followed by oxidation and N-chlorination gave other sulfone-extended solubilizers such as carboxylates **1d** (Scheme 1).

The 4-methyl-4-aminomethyl analogs were also synthesized from **3**, but displacement of the chloride was not straightforward. Reaction with dialkylamines gave two isomeric products which were separable through silica gel chromatography, namely oxazolidinones **9a–d** and 2-aminodihydrooxazoles **10a–d**. While the reaction with dimethylamine (entry **a**) has been previously re-

* Corresponding author. Tel.: +1 510 899 8871.
E-mail address: rjain@novabaypharma.com (R.K. Jain).

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ported in the literature[5], the chemical characterization was limited to IR and elemental analysis, neither of which could determine if the reported sample was a pure compound or a mixture of regioisomers (Scheme 2).

The structures of **9a** and **10a** were established by HMBC experiments. A crosspeak between the dimethyl signal and the imidate carbon atom in compound **10a** verified that the lower R_f isomer was the dihydrooxazole **10a** rather than a carbamate. Likewise, a crosspeak between the dimethyl signal and an alkyl carbon atom in compound **9a** verified that the dimethylamino group was linked through the methylene chain (Fig. 1).

We propose that compound **10a** comes from addition of the dimethylamine into the carbamate carbonyl rather than through the expected S_N2 displacement of chloride **3**.[6] The urea intermediate can then cyclize to give the isourea[7] (Scheme 3).

Addition of iodide to the reaction mixture to form the alkyl iodide in situ did improve the isolated yield of the desired oxazolidinone, but did not reduce the isolated yield of the dihydrooxazole. In addition, the isolated yields of oxazolidinone from different secondary amines was reasonably constant. However, the reaction with *tert*-butylamine caused decomposition of the starting material with neither the oxazolidinone nor the dihydrooxazole isolated (Table 1).

Oxazolidinones **9a** and **c** were further alkylated to give ammonium-solubilized oxazolidinones **11a–d** and N-chlorination gave **1e–h** (Scheme 2).

Synthesis of the 5-sulfonylmethyl analog was accomplished through the sequence shown in Scheme 4. A Henry reaction between 2-nitropropane and benzyloxyacetaldehyde gave nitro alcohol **12**, which was reduced with hydrogen to give amino alcohol **13**. Surprisingly, the benzyloxy group was not cleaved under these conditions. Cyclization to oxazolidinone **14**, debenzylation with a palladium catalyst, followed by transformation of the alcohol to the sulfonic acid **17**, through a thioacetate intermediate **16**, pro-

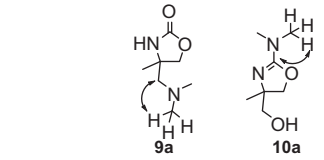
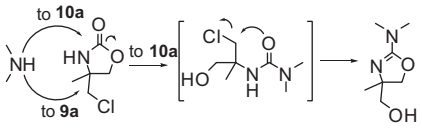


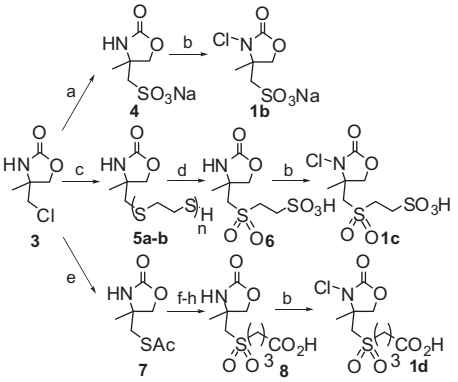
Figure 1. Non-trivial crosspeaks observed in the HMBC spectra of **9a** and **10a**.



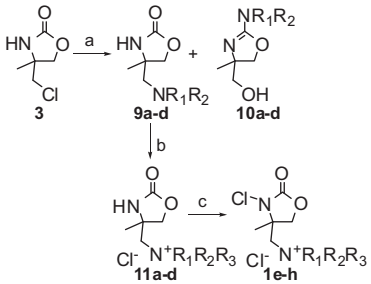
Scheme 3. Hypothesized mechanism of rearrangement to give **10a**.

Table 1
Reaction conditions to form oxazolidinones **9a–d** and dihydrooxazoles **10a–d**

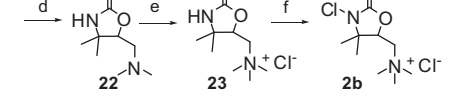
Entry	R_1, R_2	Additive	Yield (**9**) (%)	Yield (**10**) (%)
a	–Me, –Me	None	30	41
a	–Me, –Me	NaI	40	41
b	–Et, –Et	none	3	19
b	–Et, –Et	NaI	39	51
c	$-(CH_2)_4-$	NaI	35	60
d	$-(CH_2)_5-$	NaI	49	36
e	–H, –*t*Bu	None	Trace	Trace



Scheme 1. Synthesis of 4-substituted anionic oxazolidinones **1b–d**. Reagents and conditions: (a) Na_2SO_3, DMF/H_2O, 50 °C, quant.; (b) *t*-BuOCl, MeOH, 0 °C, 40–90% over 1–2 steps; (c) EDT, DMF, 90 °C; (d) mCPBA, DCM, 0 °C, 33% over two steps; (e) KSAc, DMF, 90 °C, 46%; (f) NaOH, MeOH, rt; (g) $Br(CH_2)_3CO_2Et$, DMF, 80 °C, 71% over two steps; (h) LiOH, MeOH/H_2O, rt, 18%.



Scheme 2. Synthesis of cationic oxazolidinones **1e–h**. Reagents and conditions: (a) HNR_1R_2, NaI, THF, 85 °C, 35–49%; (b) R_3I, MeOH, 25–70 °C; Ag_2O; HCl; (c) *t*-BuOCl, MeOH, 15–48% over two steps.



Scheme 4. Synthesis of 5-substituted anionic oxazolidinone derivative **2a**. Reagents and conditions: (a) TMG, THF, rt, 84%; (b) H_2, Raney Ni, MeOH, 89%; (c) triphosgene, NEt_3, DCM, 0 °C, 78%; (d) H_2, Pd/C, MeOH/THF, 99%; (e) PPh_3, DIAD, HSAc, THF, 0 °C, 87%; (f) H_2O_2, HCO_2H, rt, 90%; (g) *t*-BuOCl, MeOH, 0 °C, 53%.

Scheme 5. Synthesis of 5-substituted cationic oxazolidinone derivative **2b**. Reagents and conditions: (a) *m*CPBA, DCM, 87%; (b) $HNMe_2$, THF/H_2O, 42%; (c) H_2, Raney Ni, MeOH, 76%; (d) triphosgene, NEt_3, DCM, 0 °C, 18%; (e) MeI, MeOH; Ag_2O; HCl, 71%; (f) *t*-BuOCl, MeOH, 0 °C, 48%.

vided the 5-substituted sulfonic acid, which was *N*-chlorinated to give oxazolidinone **2a**.

Synthesis of the 5-aminomethyl derivative was accomplished through epoxidation of alkene **18**[8], followed by ring opening with dimethylamine, reduction of the nitro group, and cyclization to the oxazolidinone. Alkylation and N-chlorination afforded compound **2b** (Scheme 5).

Two des-methyl analogs of **2c** and **d** were synthesized through the route in Scheme 5. Starting with commercially available chloride **24**, the alkyl chloride was either replaced with a sulfonate (**26**) through the thioacetate **25**, or by displacement with pyridine to give its pyridinium analog **27** (Scheme 6). Unfortunately, *N*-chlorinated analogs **2c–d** were not sufficiently stable to test in an MBC assay. It is our view that the gem-dimethyl group has a stabilizing effect on the *N*-chlorooxazolidinones by either blocking dehydrochlorination (similar to the stabilizing effect of a gem-dimethyl group on *N*,*N*-dichlorotaurine[9]) or oxazolidinone hydrolysis. Further studies are expected to elucidate the importance of the influence of the 4,4-disubstitution on stability.

Increasing the steric bulk at the 4-position afforded better chemical stability than **2c–d** (data not shown). The spirocycle **28**[10] was alkylated with methyl iodide to give the spirocyclic ammonium derivative **29**; N-chlorination gave compound **2e** which showed much better stability than did **2c–d** (Scheme 7).

In vitro antimicrobial activity of analogs **1a–h** and **2a–e** (Fig. 2) was conducted against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Candida albicans* ATCC 10231 using a modified CLSI M26-A protocol where Mueller–Hinton broth was replaced with phosphate-buffered saline (pH 7) and the residence time of the compound was reduced to 1 h to generate minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC). In vitro cytotoxicity was measured against L929 (mouse fibroblast) cells after exposure to the compounds for 1 h. After exposure, the test article was removed and cells were incubated overnight. Viability was measured using the Dojindo Cell Proliferation Assay (Dojindo Laboratories, Mashiki, Kumamoto prefecture, Japan).

Antibacterial therapeutic indices were calculated based on the geometric mean of the MBCs against *E. coli* and *S. aureus*, antifungal therapeutic indices were calculated based on the MFC against *C. albicans*.

Results and discussion: We showed that by appending a water-solubilizing group to either the 4- or 5- position, a 10-

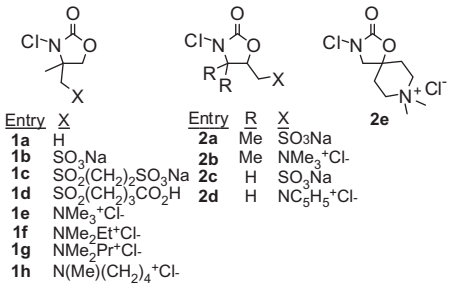


Figure 2. Reported compounds.

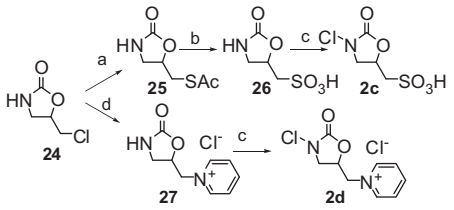
to 50-fold reduction in cytotoxicity results. Also, up to 64-fold increase in antifungal activity is observed, and the antibacterial activity was retained with this added functionality (Table 2, entry **1g**).

Compared to parent oxazolidinone **1a**, sulfonate-solubilized **1b** showed a 15-fold reduction in in vitro cytotoxicity with a corresponding reduction of activity reflected against bacteria and fungi, with 2- to 256-fold reductions in antimicrobial activity. Somewhat surprisingly, extending the water-solubilizing group with a sulfone (**1c–d**) increased the antimicrobial activity relative to **1b** without significantly affecting the CT_{50}.

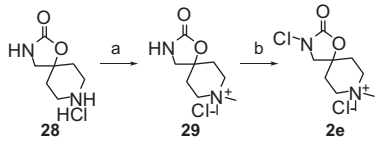
Table 2
Antimicrobial activities, cytotoxicities, and calculated in vitro therapeutic indices against bacteria and fungi for reported compounds

Entry	*E. coli*	*S. aureus*	*C. albicans*	CT_{50}	$TI_{E.\ coli/S.\ aureus}$	$TI_{C.\ albicans}$
1a	1	4	512	0.07	5.2	0.02
1b	256	128	1024	0.8	1.4	0.25
1c	16	32	128	0.6	8.5	1.5
1d	16	8	>1024	0.52	14	0
1e	2	4	16	n.d.	n.d.	n.d.
1f	2	4	8	0.86*	78	28
1g	2	2	8	0.9*	122	31
1h	4	4	16	1.13*	76	19
2a	256	512	1024	4.3	2.9	1.0
2b	16	32	64	0.69	7.6	2.7
2e	16	32	256	0.7*	7.9	0.70

Antimicrobial activities (MBCs and MFCs) are given in μg/ml, CT_{50}s are given in mM, n.d. = not determined, * = Testing solution was pH 4 rather than pH 7. Results for other compounds (data not shown) show that CT_{50} values are comparable at the two pHs.



Scheme 6. Synthesis of 4,4-unsubstituted oxazolidinones **2c**–**d**. Reagents and conditions: (a) KSAc, DMF, 50 °C, 53%; (b) H_2O_2, HCO_2H, quant.; (c) *t*-BuOCl, MeOH, 0 °C, 35–40%; (d) pyridine, 115 °C, 36%.



Scheme 7. Synthesis of spirocycle oxazolidinone **2e**. Reagents and conditions: (a) MeI, Cs_2CO_3, DMF; Ag_2O; HCl, 92%; (b) *t*-BuOCl, MeOH, 0 °C, 13%.

Most remarkably, cationic groups (**1e–h**) showed greatly improved antifungal activity while the cytotoxicity was only slightly worse than sulfonate **1b**. The most potent antifungal compounds, **1f–g**, were 64-fold better than the parent **1a**.

This trend was also shown for 5-substituted oxazolidinones **2a–b, e**. Compared to parent **1a**, the 5-sulfonylmethyl derivative **2a** showed significantly reduced cytotoxicity, but with the cost of reduced antimicrobial activity. Replacing the sulfonate solubilizer with a cationic group (**2b**, **e**) yielded more potent antimicrobial activity (16-fold across both bacteria and fungi) at only a 7-fold cost in cytotoxicity relative to the sulfonate.

Therapeutic indices (ratios of activity to toxicity) were calculated for bacteria as well as fungi. The bacterial therapeutic index (mathematically defined as CT_{50}/MBC), increased 0- to 20-fold across the series, while the fungal therapeutic index (CT_{50}/MFC) increased 12- to 600-fold across the series (with the exception of compound **1d** which did not show antifungal activity). This remarkable transformation of compound **1a** (whose threshold for antifungal activity is 50-fold higher than its threshold for cytotoxicity) to compound **1g** (whose threshold for antifungal activity is

now 30-fold lower than its threshold for cytotoxicity) validates this approach for making safer, more potent antimicrobial agents. Research is ongoing to further elucidate the SXR by careful control of the physiochemical properties. These data together with our increasing understanding of the mechanism of action of these and similar agents should result in new safe and effective human and animal antimicrobials.

Conclusions: Changing the electronic structure around the N–Cl bond greatly affects the antimicrobial activity as well as the cytotoxicity. This offers the opportunity to better control the processes involved. This finding also supports our general assertion that these molecules are not non-specific chlorinating agents, but rather that they are capable of hitting multiple chemical targets with some degree of selectivity which ultimately results in microbial death.

The use of charged functionality for water solubilization of *N*-chlorooxazolidinones is a simple and effective tool for reducing the cytotoxicity of powerful antimicrobial agents such as 4,4-dimethyl-3-chlorooxazolidin-2-one. Addition of trialkylammonium solubilizers at either the 4- or 5- positions both increases the antifungal activity as well as reduces cytotoxicity as compared to unsolubilized oxazolidinones, thereby greatly increasing their therapeutic index.

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