MEDI 300

N-Chlorooxazolidin-2-ones as Antimicrobial Agents at Neutral pH

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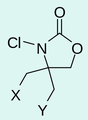
Abstract

Previously synthesized *N*-chloramines have excellent activity at low pH; however, they can display reduced activity at physiological pH. Our recent structure/stability/activity relationship efforts enabled identification of new *N*-chlorooxazolidinones as agents with improved antimicrobial activity under a broad pH range. Syntheses and *in vitro* antimicrobial activity of new 5-membered heterocycles are discussed, demonstrating potent antimicrobial compounds with bactericidal activity (1 hr MBC) against *Staphylococcus aureus* and *Escherichia coli* at 0.5 µg/mL and fungicidal activity (1 hr MFC) against *Candida albicans* as low as 8 µg/mL at pH 7.



X = H; Y = F: MBC(*E.coli, pH7*)= 0.5 µg/ml
X = H; Y = Cl: MBC(*E.coli, pH7*)= 0.75 µg/ml
X= Cl; Y = Cl; MBC(*E.coli, pH7*)= 1 µg/ml

Introduction

Bacteria are becoming resistant to currently available antibiotics, driving the need for new antimicrobial agents with novel mechanisms of action and low potential for the development of resistance. *N*-chlorotaurine **1** and *N,N*-dichlorotaurine **2** are endogenous antimicrobials that are generated in the oxidative burst of activated neutrophils and macrophages. They are utilized by the innate immune system to destroy invading pathogens and have shown minimal potential for developing resistant bacteria.[1,2] Sulfonic acids **3** and **4** have been designed to possess improved aqueous stability over the parent analogs, **1** and **2**. Compound **3** is currently being evaluated as a clinical candidate for several topical applications.[3] Nonetheless, compounds with increased activity over a range of pH's would have utility for therapeutic indications that necessitate application to physiologically buffered fluids.

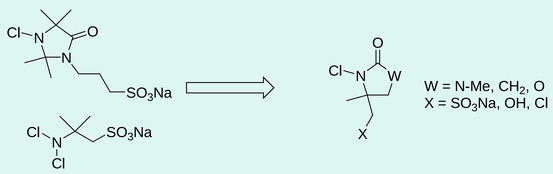
1: R = H
2: R = Cl

3

4

pH Dependant Activity of Chloramines

Mono- and di-chloramine analogs of endogenous chlorinated taurines, **3** and **4**, display pH dependent anti-microbial activity with increased killing at lower pH. In part, the changes in biological activity between pH 4 and 7 are attributed to increasing reactivity rates of these compounds with nucleophiles under the more acidic conditions, as has been reported with **1**.[4] In order to broaden the utility of this class of antimicrobials, we endeavored to design compounds with improved activity at neutral pH. *N*-chlorinated heterocycles that contain electron withdrawing groups adjacent to the chlorinated nitrogen were investigated. By reducing the electronegativity of the nitrogen, we envisioned minimizing potential protonation at rate limiting steps to mitigate the pH dependence typically observed in aliphatic chloramines.



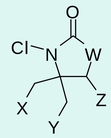
W = N-Me, CH_2, O
X = SO_3Na, OH, Cl

Initial investigations into heterocyclic chloramines had shown that sulfonic acid functionalized 2-imidazolidinones **5**, pyrrolidones **10**, and oxazolidinones **12** were aqueous stable compounds with poor antimicrobial activity at both pH 4 and pH 7.[5] Unlike previous series,[5] the oxazolidinone **11** showed pH independent biological activity. In addition, based on earlier experience, we had expected that the activity of lipophilic oxaloidinone analogs, such as **11**, to be comparable to the sulfonic acid **12**. But in fact, when the solubilizing group X was modified to a less polar hydroxyl or chloro group, a substantial increase in anti-bacterial activity was observed for both pHs.

Oxazolidinones as Activated Heterocycles

The oxazolidinone series showed the greatest activity of the three heterocyclic cores tested, with sub µg/ml activity for both pH's in the chloro analog **11**. In addition, unlike the chloloramine series, the safety profile of these compounds, as estimated by the ratio of the compounds' cytotoxicity (CT_{50} against L929 cells) *vs.* MBC, did not substantially decrease with more lipophilic analogs. Additional modifications incorporating neutral, electron withdrawing functionality at the 4 and 5 positions of the oxazolidinone ring were tested. The electronic effects of these substituents had little effect on the aqueous stability or activity. Instead a wide range of lipophilic analogs showed potent anti-bacterial activity against Gram-negative and Gram-positive examples.

Biological Assay Results



Compounds 5-23.

CMPD	W	X	Y	Z	MBC or MFC (µg/ml)[a]						Aq. Stability @ pH7 40°C	CT_{50}/ MBC[b]
					E. coli ATCC 25922		*S. aureus* ATCC 29213		*C. albicans* ATCC 10231			
					pH 4	pH 7	pH 4	pH 7	pH 4	pH 7	$t_{1/2}$ (days)	
3	-	-	-	-	2	256	2	256	32	2048	>249	5.7
4	-	-	-	-	4	128	2	256	16	256	>249	12
5	N-Me	H	OH	H	16	8	16	32	8	>1024	>158	7.3
6	N-Me	H	Cl	H	8	8	32	16	32	512	>92	1
7	N-Me	H	SO_3Na	H	>256	>1024	>256	>1024	>256	>512	>100	< 0.2
8	CH_2	H	OH	H	32	8	128	128	>256	>512	>159	8.6
9	CH_2	H	Cl	H	8	2	32	128	>256	>512	>159	3.2
10	CH_2	H	SO_3Na	H	256	>1024	128	>2048	>256	512	>256	< 5.4
11	O	H	Cl	H	0.25	0.75	0.25	0.5	>128	>128	40	41
12	O	H	SO_3Na	H	256	128	256	256	>512	1024	140	1.4
13	O	H	Br	H	0.25	0.5	0.5	1	>256	64	5	45
14	O	H	SO_2Et	H	0.5	2	0.5	2	>256	64	20	31
15	O	H	SO_2Pent	H	0.5	4	0.5	1	>256	>156	18	-
16	O	H	F	H	0.25	0.5	0.5	0.5	>256	32	>28	-
17	O	H	CF_3	H	0.5	4	2	4	16	>1024	>43	-
18	O	-butyl-		H	1	2	2	2	128	64	70	-
19	O	Cl	SO_3Na	H	32	8	128	16	>256	64	16	-
20	O	Cl	SO_2EtSO_3Na	H	4	4	32	4	128	8	5	-
21	O	Cl	Cl	H	0.063	1	0.063	0.5	128	16	28	87
22	O	H	H	CH_2OH	2	2	4	4	>512	1024	28	13
23	O	H	H	CH_2Cl	0.5	2	0.5	1	>256	512	194	0.1

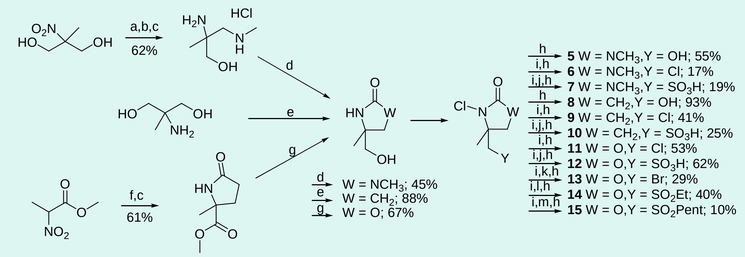
[a] MBC is determined using a modification of a standard method described in CLSI M26-A where Mueller-Hinton broth is replaced by isotonic saline at pH 4 or 7 and the assay is performed for 1 hour at room temperature. [a] *S. aureus* MCC 91731: b. *E. coli* MCC 80392 : [c] *C. albicans* MCC 50319.

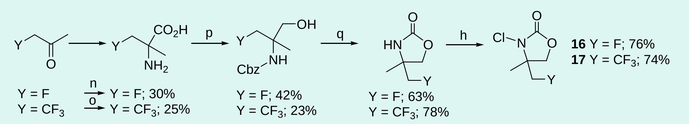
[b] Safety profile is calculated by the ratio of CT_{50} against L929 cells *vs.* 1 hr MBC as measured against *E. coli* at pH 7.

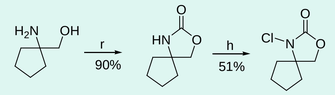
References

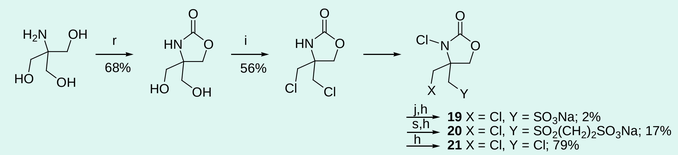
1) Nagl, M.; Hess, M. W.; Pfaller, K.; Hengster, P.; Gottardi, W. *Antimicrob. Agents Chemother.* **2000**, *44,* 2507-2513. **2)** Nagl, M.; Gottardi, W. *Hyg. Med.* **1996**, *21, 597-605.* **3)** Wang, L.; Khosrovi, B.; Najafi, R. *Tetrahedron Lett*. **2008**, *49*, 2193–2195. **4)** Calvo, P.; Crugeiras, J.; Rios, A.; Rios. MA. *J. Org. Chem.* **2007**, *72*, 3171-3178. **5)** a) Shiau T.; Turtle E; Francavilla C.; Alverez N.; Zuck M.; Friedman L.; O'Mahony D.; Low E.; Anderson M.; Najafi R.; Jain R. *Bioorg. Med Chem. Lett.* **2011**, *21*, 3025-3028. b) Francavilla, C.; Turtle E.; Kim B.; O'Mahony D.; Shiau T.; Low E.; Alverez N.; Celeri C.; D'Lima L.; Friedman L.; Ruado F.; Xu P.; Zuck M.; Anderson M.; Najafi R.; Jain R. *Bioorg. Med Chem. Lett.* **2011***, 21*, 3029-3033.

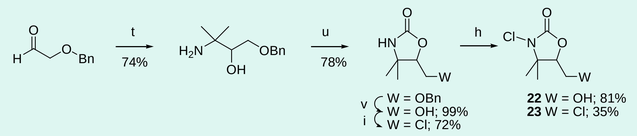
Synthesis of N-Cl-Heterocycles











Reagents and conditions: (a) $CH_2O_{(aq)}$, $MeNH_2$, 48h; (b) HCl, EtOH, H_2O, reflux, 5h; (c) Raney-Ni, H_2, MeOH, 18h; (d) urea (melt), 200˚C, 1h; (e) Diethyl carbonate (neat), 140˚C, 6h; (f) methyl acrylate, TEA, THF, 0-25˚C, 16h; (g) $NaBH_4$, EtOH, 21h; (h) *tert*-butyl hypochlorite, MeOH, 0-25˚C, 1-3h; (i) $SOCl_2$, Pyr, DCE, reflux, 4h; (j) $NaSO_3$, DMF, H_2O, 60°C; (k) NaBr, DMF, 80˚C, 6h.; (l) Ethanethiol, K_2CO_3, MeOH, 24hr; (m) pentanethiol, K_2CO_3, MeOH, 24h; (n) i. $NH_4Cl_{(aq)}$, KCN, 16h; ii. HCl, reflux, 16h; (o) i. *t*-$BuSONH_2$, $Ti(OEt)_4$, THF, reflux, 16h; ii. Et_2AlCN, *i*-PrOH, THF -78 to 25˚C, 16h; iii. HCl, 100˚C, 24h; (p) i. CbzOSu or CbzCl, TEA, MeOH, 16h; ii. Ethyl chloroformate, TEA, THF; $NaBH_4$, 0-25˚C; (q) NaH, THF, 3h; (r) i. Ethyl chloroformate; ii. NaOH, 0-25˚C; (s) i. $NaSCH_2CH_2SO_3Na$, $CsCO_3$, DMF, rt, 18h; ii. H_2O_2, HCO_2H, rt, 18h; (t) i. 2-Nitropropane, TMG, THF, 18h; ii. Raney-Ni, H_2, MeOH, 24h; (u) Triphosgene, TEA, DCM, 0˚C-rt, 5h; (v) Pd/C, H_2, MeOH, 5 days.

Conclusions

- *N*-Chlorinated oxazolidin-2-ones with lipophilic sidechains, such as **11**, **13**, **16**, and **21** have excellent activity against *E. coli* and *S. aureus* at both pH 7 and 4, and the safety profile of these compounds at pH 7, as measured by the ratio of the CT_{50}(L929) *vs.* 1 h MBC (*E.coli*), is comparable to more polar analogs, differentiating this class from previous di- and mono-chloramine series.
- Compounds **16**, **20**, and **21** show good anti-fungal activity at pH 7 against *C. albicans,* and compound **16** has very good safety in the CT_{50} cell assay.
- Compounds **11**, **16**, and **21** have bactericidal and fungicidal activity and are potential advanced candidates suitable for IND-enabling studies.