MEDI 302

Quaternary ammonium stabilized dichloroamines as antimicrobial agents

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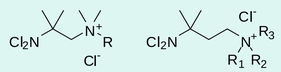
Abstract

N,N-Dichlorotaurine and *N*-chlorotaurine are potent, broad-spectrum antimicrobial agents produced by neutrophils during phagocytosis. Their use as a therapeutic agent is only limited due to their short shelf-life in solution at room temperature. Our structure activity/stability relationship study identified compounds **1** and **2** as water stable dichloroamines that retain excellent *in vitro* activity and good solution stability. A medicinal chemistry approach to improve the antimicrobial activity while maintaining aqueous stability of this class of agents will be examined. These analogs depict a profile of bactericidal activity (1 hr MBC) against *Staphylococcus aureus* and *Escherichia coli* in the range of 2-2048 µg/mL at both pH 4 and pH 7 with several analogs exhibiting potent activity. Furthermore, some analogs had fungicidal activity (1 hr MFC) against *Candida albicans* in the range of 4-2048 µg/mL at pH 7.



1a-b **2a-n**

Introduction

Many antimicrobial compounds used for the prevention or treatment of infections have been rendered less effective through evolved bacterial drug resistance. This has engendered both an urgent need and widespread interest in new, fast-acting, broad spectrum, topical antimicrobials with reduced potential for inducing resistance.

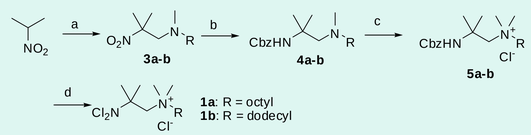
The chlorinated derivatives of taurine, *N*-chlorotaurine and *N,N*-dichlorotaurine are part of the innate mammalian response to infection, produced as antimicrobials to destroy invading microorganisms and protect the body. Myeloperoxidase (MPO; EC 1.11.1.7) in human granulocytes and monocytes uses hydrogen peroxide and chloride to generate hypochlorous acid, which reacts with taurine to produce the longer lived antimicrobial *N*-chlorotaurine.

N-Chlorotaurine has evolved as a natural antimicrobial compound with no known bacterial resistance, and provides a unique starting point for novel antibiotic drug development. Nagl et al., have previously reported the anti-microbial activity of *N*-chlorotaurine. However, its commercial utility may be limited due to its short shelf-life in solution. Fortunately, we have determined that key properties of chloramine-based antimicrobials can be tailored through the molecule's core targeting moiety (CTM), allowing us to regulate the biological activity, toxicity, and physiochemical properties such as reactivity, stability, and solubility.

These *N,N*-dichloramines are a promising class of antimicrobials with a unique mechanism of action (MOA). We are in the process of elucidating the MOA and our studies so far indicate a very rapid inactivation of sulfur containing proteins resulting in their dysfunction and dysregulation leading to the death of the pathogen.

One-methylene Spacer

2-Nitropropane was condensed with formaldehyde and an amine under Mannich conditions to afford nitro-amines **3a-b**. Difficulty in quaternization under standard conditions necessitated the reduction of the nitro, and then reprotection of the resulting amine to give Cbz-amines **4a-b**, which could then be quaternized. Deprotection of the amine and chlorination afforded compounds **1a-b**.

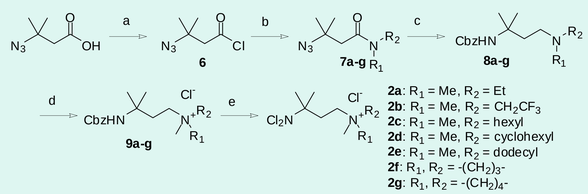


1a: R = octyl
1b: R = dodecyl

Conditions: (a) CH_2O, MeHNR, *i*-PrOH/H_2O; (b) H_2, Raney Ni, MeOH; then CbzOSu, THF/H_2O; (c) MeI, EtOH, 50ºC; then Ag_2O, AcOH; then HCl, H_2O; (d) H_2, Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Two-methylene Spacer

Amidation of acid chloride **6** gave amides **7a-g**. Reduction of the azide and reprotection of the resulting amine afforded Cbz-protected tertiary amines **8a-g**. The tertiary amines were quaternized with methyl iodide to afford Cbz-protected quaternary amines **9a-g**. Removal of the Cbz group and chlorination afforded compounds **2a-g**.

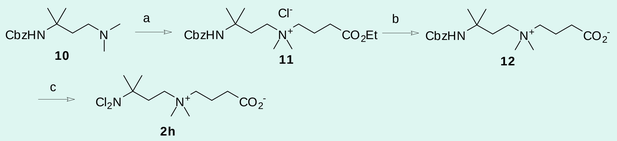


2a: R_1 = Me, R_2 = Et
2b: R_1 = Me, R_2 = CH_2CF_3
2c: R_1 = Me, R_2 = hexyl
2d: R_1 = Me, R_2 = cyclohexyl
2e: R_1 = Me, R_2 = dodecyl
2f: R_1, R_2 = -$(CH_2)_3$-
2g: R_1, R_2 = -$(CH_2)_4$-

Conditions: (a) $SOCl_2$, DCE, 50ºC; (b) HNR_1R_2, DCE, DIEA, 0ºC; (c) $LiAlH_4$, THF, reflux; (d) CbzOSu, $NaHCO_3$, THF/H_2O; (e) MeI, EtOH, 50 ºC; then Ag_2O, AcOH; then HCl, H_2O; (f) H_2, Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Carboxylate-solubilized alkyl group

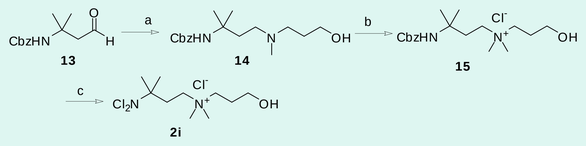
Quaternization of previously reported intermediate **10** afforded ester **11**, which was then hydrolyzed to give zwitterion **12**. Removal of the Cbz group and chlorination of the resulting amine afforded compound **2h**.



Conditions: (a) $Br(CH_2)_3CO_2Et$, 1,4-dioxane, 95ºC; (b) NaOH, MeOH; (c) H_2, Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Alcohol-solubilized alkyl group

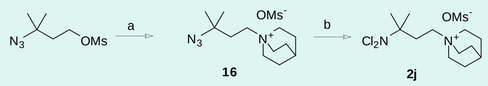
Intermediate **14** was synthesized from the reductive amination of compound **13**, whose synthesis is described further below. The resulting tertiary amine was quaternized, deprotected, and chlorinated to give compound **2i**.



Conditions: (a) $MeHN(CH_2)_3OH$, $NaBH(OAc)_3$, DCE; (b) MeI, EtOH, 50 ºC; then Ag_2O, AcOH; then HCl, H_2O; (c) H_2, Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Quinuclidinyl Analog

Quaternary amines could also be synthesized from the alkylation of nucleophilic amines such as quinuclidine, which displaced a mesylate to give azide-ammonium **16**. The azide was reduced and the resulting amine chlorinated to give compound **2j**.

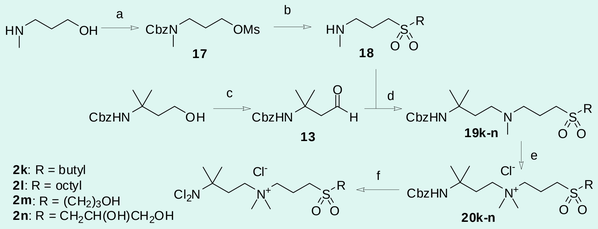


Conditions: (a) $N(CH_2CH_2)_3CH$, 3-pentanone, 110 ºC; (b) H_2, Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Sulfone-extended alkyl chains

While the long-chain ammonium **2e** was extremely potent in antimicrobial assays, the solubility of the compound suffered due to the extreme hydrophobicity of the molecule. Desire for substitution of the long chain with more polar, yet hydrophobic, groups led us to sulfone-extended ammonium compounds **2k-n**. First, 3-methylamino-1-propanol was *N*-protected with a Cbz group and *O*-activated for displacement by mesylation (17). Displacement of the mesylate with a thiol, followed by oxidation to the sulfone, and deprotection of the Cbz group afforded amines **18k-n**.

3-(Carbobenzyloxy)amino-3-methyl-1-butanol was oxidized with PDC to give the resulting unstable aldehyde which was immediately coupled with the amines by reductive amination. The resulting tertiary amines were then quaternized and chlorinated to give compound **2k-n**.



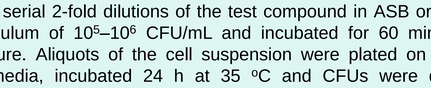
2k: R = butyl
2l: R = octyl
2m: R = $(CH_2)_3OH$
2n: R = $CH_2CH(OH)CH_2OH$

Conditions: (a) CbzOSu, DCE; then MsCl, TEA, DCE; (b) HSR, Cs_2CO_3, DMF; then H_2O_2, HCO_2H; then H_2, Pd/C, MeOH; (c) PDC, NaOAc, DCM; (d) $NaBH(OAc)_3$, DCE; (e) MeI, EtOH, 50 ºC; then Ag_2O, AcOH; then HCl, H_2O; (d) H_2, Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Biological Activity

Prior to antimicrobial testing, all compounds were screened for compound stability. In several cases (**1a-b**, **2m**), compounds were unstable in aqueous solution and were not tested for *in vitro* antimicrobial activity.

Susceptibility testing of *Escherechia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 to **2a-l** and **2n** was conducted using modified CLSI methods as described previously. Briefly, CLSI M26-A protocol for Minimum Bactericidal Concentration (MBC) testing was modified by substituting 0.9% acetate saline buffer, pH4 (ASB) or phosphate-buffered saline; pH 7(PBS) for Cation-Adjusted Mueller Hinton Broth (CAMHB) to circumvent for the reactivity of chlorine to certain components of CAMHB. Due to the rapid cidal effect of the compounds, the MBC assay was shortened from 16–20 hours at 35 ºC to 60 min at room temperature. The MBC was defined as the lowest concentration achieving >99.9% kill. Microorganisms were first grown to mid-log phase, centrifuged and suspended in ASB or PBS. Next, the organisms were added to serial 2-fold dilutions of the test compound in ASB or PBS to a final inoculum of 10^5–10^6 CFU/mL and incubated for 60 min at room temperature. Aliquots of the cell suspension were plated on agar with growth media, incubated 24 h at 35 ºC and CFUs were quantified.



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2e: R_1 = Me, R_2 = dodecyl
2f: R_1, R_2 = -$(CH_2)_3$-
2g: R_1, R_2 = -$(CH_2)_4$-
2h: R_1 = Me, R_2 = -$(CH_2)_3CO_2^-$
2i: R_1 = Me, R_2 = -$(CH_2)_3OH$
2k: R_1 = Me, R_2 = -$(CH_2)_3SO_2C_4H_9$
2l: R_1 = Me, R_2 = -$(CH_2)_3SO_2C_8H_{17}$
2m: R_1 = Me, R_2 = -$(CH_2)_3SO_2(CH_2)_3OH$
2n: R_1 = Me, R_2 = -$(CH_2)_3SO_2CH_2CH(OH)CH_2OH$

	pH 4				pH 7			
Entry	$t_{1/2}$ (days)	MBC *E. coli* (ug/ml)	MBC *S. aureus* (ug/ml)	MFC *C. albicans* (ug/ml)	$t_{1/2}$ (days)	MBC *E. coli* (ug/ml)	MBC *S. aureus* (ug/ml)	MFC *C. albicans* (ug/ml)
1a	1				0			
1b	0				0			
2a	>147	4	2	>1024	50	512	>512	>1024
2b	89	4	2	32	25			
2c	33	4	2	>1024	28	128	256	>1024
2d	20	16	4	>1024	27			
2e	19	4	2	8	17	8	4	4
2f	107	4	4	>1024	>35	256	1024	>1024
2g	90	4	2	32	75			
2h	100	4	2	32	48	512	>2048	>1024
2i	>169	4	4	64	70	1024	>2048	>1024
2j	107	8	2	32	>107			
2k	>28	8	2	16	>28	512	1024	>1024
2l	160	4	4	32	120	64	128	256
2m	0				0			
2n	>28	8	4	32	>28	2048	>2048	>2048

blank = not tested due to compound stability

Conclusions

- Long-chain, lipophilic dichloroamines are potent bactericidal and fungicidal compounds at pH 4.
- Compound **2e** is bactericidal and fungicidal at single-digit ug/ml at pH 4 and pH 7 and is suitable for applications in hydrophobic formulations.
- Substitutions of the alkyl chain with a sulfone group increases the water solubility..