Poster # 81

New *N*-Chloroamines: Chemical Synthesis, Solution Stability and Biological Activity

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Abstract

2-Dichloroamino-2-methyl-propane-1-sulfonic acid sodium salt (1) is currently a clinical candidate as a topical antimicrobial agent. Structure-activity relationships of its analogs were explored to achieve optimal antimicrobial activity with minimal mammalian toxicity while maintaining aqueous stability. All the analogs synthesized showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in the range of 2- >256 µg/mL and cytotoxicity against mammalian L929 cells in the range of 0.03-5.8 mM. After systematically varying the distance between the dichloroamine and other functional groups, the ideal distances were determined for optimum aqueous stability and biological activity. This study has led to the identification of a new class of solution stable topical antimicrobial agents which have sulfones in the backbone (2) and quaternary ammonium salts (3) as water solubilizing groups.

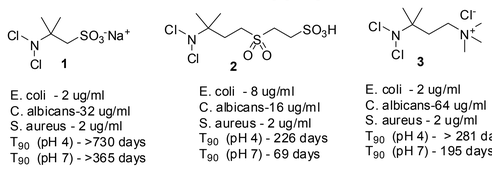


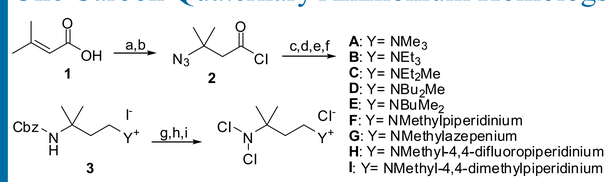
Figure 1. Promising compounds from SSR/SAR studies in *N,N*-dichloroamine series

Introduction

Bacteria are becoming resistant to most currently available antibiotics. To overcome this problem an antimicrobial agent with a novel mechanism of action and low potential for the development of resistance is desired. *N*-chlorotaurine, a molecule in neutrophils, has no reported incidence of developed resistance. Nagl[1] *et al* have previously reported the bactericidal, fungicidal and virucidal activity of *N*-chlorotaurine. Its therapeutic utility, however, is limited due to its poor long-term solution stability at room temperature.[2] In our ongoing program to develop topical antimicrobial compounds[3] we explored structure stability relationship (SSR)/SAR in dichloroamine compounds to identify drug like molecules.

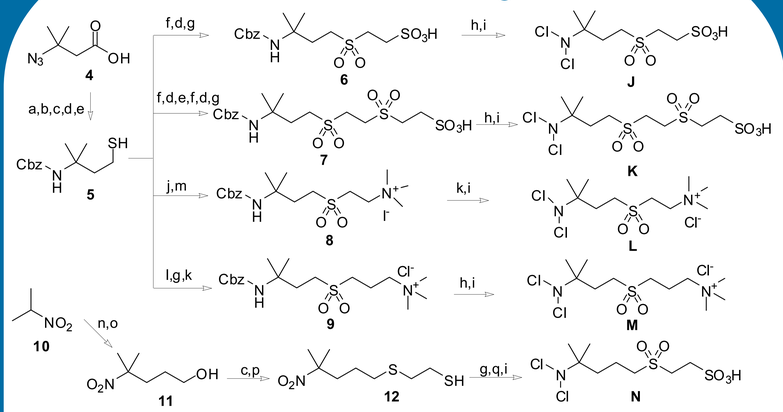
In our previous work we substituted the sulfonic acid with other water solubilizing group (WSG) as well as incorporated functional groups into the backbone that provided aqueous stability comparable to **1**.[4] We further explored the chain length between the quaternary carbon and the WSG or a functional group in the backbone between the WSG and the dichloroamine. The syntheses of the one carbon homologs with trialkyl or cyclic ammonium salts are presented in Scheme 1. The ammonium function acts as both a dichloroamine stabilizing group and the WSG in these analogs. Our earlier backbone modification studies suggested that sulfone analogs showed the best solution stability. The syntheses of sulfone analogs with various WSGs are presented in Scheme 2. Analogs with one or two sulfone repeat units and a sulfonic acid were prepared to examine the effect of lipophilicity on solution stability. The syntheses of two carbon homologs with various WSGs helps us to determine the importance of chain length and WSG towards solution stability is presented in Scheme 3. A zwitterionic compound was synthesized to isolate factors for stability from water solubilizing ability of an ammonium function in Scheme 4.

One Carbon Quaternary Ammonium Homologs



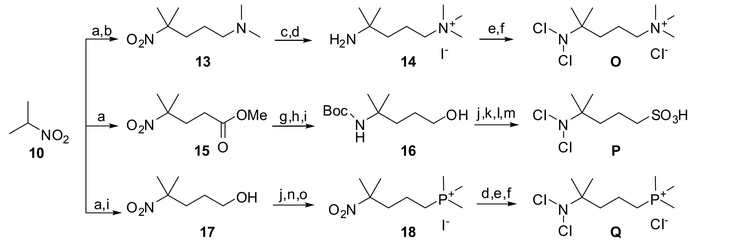
Scheme 1. Reagents and conditions: (a) NaN_3, AcOH, H_2O, 95 °C, 48 h; (b) $SOCl_2$, DCE, reflux, 4 h; (c) Secondary amines (dimethylamine for A) or (diethylamine for B and C) or (dibutylamine for D) or (*N*-methylbutan-1-amine for E) or (piperidine for F) or (azepane for G) or (4,4-difluoropiperidine for H) or (4,4-dimethylpiperidine for I), DCM, ice-cooled, 2 h; (d) LAH, THF, 70 °C, 8 h; (e) THF, Cbz-OSu, 20 °C, 16 h; (f) MeI (for A,C-I) EtI (for B), EtOH, 20 °C, 16 h; (g) Ag_2O, water, 0.5 h; then aq. HCl; (h) 10% Pd-C, MeOH, H_2, 20 °C, 16 h; (i) MeOH, *t*-BuOCl, 0-20 °C, 1 h.

Sulfone Analogs



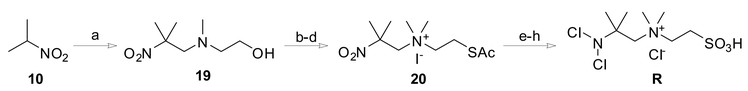
Scheme 2. Reagents and conditions: (a) LAH, ether, 0-25 °C, 16 h; (b) Cbz-OSu, isopropanol-H_2O, 16 h; (c) $MeSO_2Cl$, CH_2Cl_2, Et_3N, 0 °C, 2 h; (d) KSAc, DMF, 20-70 °C, 16 h; (e) MeOH, aq. NaOH, 1 h; (f) 1-bromo-2-chloroethane, Cs_2CO_3, DMF-Water, 20 °C, 16 h; (g) HCO_2H, 30% H_2O_2, 20 °C, 2 h; (h) 10% Pd-C, MeOH, H_2, 20 °C, 16 h; (i) *t*-BuOCl, 0-10 °C, 1 h; (j) 2-bromo-*N,N,N*-trimethylethan-1-ammonium bromide, Cs_2CO_3, DMF-Water, 20 °C, 16 h; (k) Ag_2O, AcOH, water, 0.5 h; then aq. HCl; (l) 3-bromo-*N,N,N*-trimethylpropan-1-ammonium bromide, Cs_2CO_3, DMF-Water, 20 °C, 16 h; (m) 33% HBr AcOH; (n) methyl acrylate, $BnN^+Me_3OH^-$, dioxane, 100 °C, 1 h; (o) $LiBH_4$, MeOH, 20 °C, 16 h; (p) Sodium 2-mercaptoethanethiolate, EtOH, 90 °C, 0.5 h; (q) Raney Ni, H_2 (500 psi), MeOH-H_2O, 20 °C, 72 h.

Two Carbon Homologs



Scheme 3. Reagents and conditions: (a) *N,N*-dimethylacrylamide or methyl acrylate, $BnN^+Me_3OH^-$, dioxane, 100 °C, 1 h; (b) BH_3.THF, 70 °C, 2 h; (c) MeI, MeOH, 20 °C, 48 h; (d) Raney Ni, H_2 (500 psi), MeOH-H_2O, 20 °C, 72 h; (e) Ag_2O, aq. HCl, 1 h; (f) MeOH, *t*-BuOCl, 0-10 °C, 1 h; (g) 10% Pd-C, AcOH, H_2, 20 °C, 48 h; (h) Boc_2O, Et_3N, CH_2Cl_2, 20 °C, 16 h; (i) $LiBH_4$, MeOH, 20 °C, 16 h; (j) $MeSO_2Cl$, CH_2Cl_2, Et_3N, 0 °C, 2 h; (k) 4M HCl-dioxane, 20 °C, 16 h; (l) 1M Na_2SO_3, 20 °C, 16 h; (m) Aq HOCl, 0-20 °C, 1 h; (n) NaI, acetone, 20 °C, 16 h; (o) PMe_3, THF, 100 °C, 96 h.

Zwitterion Analog



Scheme 4. Reagents and conditions: (a) 2-(Methylamino)ethanol, HCHO, NaOH, isopropanol-water, 20 °C, 16 h; (b) $MeSO_2Cl$, CH_2Cl_2, Et_3N, 0 °C , 2 h; (c) KSAc, ACN, 50 °C, 4 h; (d) MeI, EtOH, 45 °C, 16 h; (e) HCO_2H, 30% H_2O_2, 20 °C, 17 h; (f) Ag_2O, water then aq. HCl; (g) Raney Ni, H_2 (500 psi), MeOH-H_2O, 20 °C, 17 h; (h) MeOH, *t*-BuOCl, 0-10 °C, 1 h.

Table 1. Biological activity and solution stability of 1 and its analogs

Compds (NVC#)	m	X	n	W	MBC or MFC (µg/mL)[a] *S. aureus* ATCC 29213	*E. coli* ATCC 25922	*C. albicans* ATCC 10231	$t_{1/2}$ pH 4 (saline) (days)	$t_{1/2}$ pH 7 (phosphate) (days)
1	1	n/a	0	SO_3H	2	2	32	>730	>300
A	2	n/a	0	NMe_3	2	2	64	>281	>210
B	2	n/a	0	NEt_3	2	4	16	>173	>173[b]
C	2	n/a	0	NEt_2Me	2	8	16	>180	>180[b]
D	2	n/a	0	NBu_2Me	4	16	n/a	178	97
E	2	n/a	0	$NBuMe_2$	2	8	64	107	107[b]
F	2	n/a	0	Me-piperidinium	4	2	32	~90	>239
G	2	n/a	0	Me-azepanium	4	4	16	>98	>98[b]
H	2	n/a	0	Me-4,4-difluoro-piperdinium	2	4	32	>170	>170[b]
I	2	n/a	0	Me-4,4-dimethyl-piperidinium	4	16	n/a	>107	95[b]
J	2	SO_2	2	SO_3H	2	8	16	>342	>342
K	2	SO_2CH_2 CH_2SO_2	2	SO_3H	4	2	32	>73	~14[b]
L	2	SO_2	2	NMe_3	2	8	32	6	0
M	2	SO_2	3	NMe_3	2	8	32	>160	70[b]
N	3	SO_2	2	SO_3H	2	8	128	45	45[b]
O	3	n/a	0	NMe_3	2	8	256	~80	56
P	3	n/a	0	SO_3H	8[c]	8[d]	>128[e]	16	N/A
Q	3	n/a	0	PMe_3	4	2	>256	46	26
R	1	NMe_2	2	SO_3H	16	64	128	>271	19
S[4a]	1	SO_2	2	SO_3H	4	8	64	>93	1
T[4c]	1	n/a	0	NMe_3	16	8	128	>325	>396
U[4a]	2	n/a	0	SO_3H	4	4	16	174	111

[a] Minimum Bactericidal Concentration (MBC) was determined using a modified standard method described in CLSI M26-A whereby isotonic saline at pH 4 is substituted for Mueller-Hinton broth (MHB) to compensate for the reactivity of chlorine to certain components of MHB. Due to the rapid cidal nature of chlorinated derivatives, the assay was shortened from 16 – 20 h at 35 °C to 1 h at room temperature; [b] In 0.6% borate buffer pH 7; [c] *S. aureus* MCC 91731; [d] *E. coli* MCC 80392; [e] *C. albicans* MCC 50319; * In 5mM acetate pH4.

Results and Conclusion

The data in Table 1 summarize the antimicrobial activity for all analogs with sufficient aqueous solution stability (>24 h at room temperature). The analogs are active against all organisms tested, with no significant difference between the *in vitro* activities for gram-positive versus gram-negative organisms. Activity against *C. albicans* was the most variable for the compounds tested, ranging from 16 µg/mL in the case of **B**, **C**, **G**, **J** to greater than >128 µg/mL for compound **P** and **Q**. Comparison of the stability of the sulfone sulfonic acids at pH 4 and 7 had a trend of **S** < **N** < **J**, where the one carbon homolog was the most stable. Comparison of the stability of the sulfonic acids only at pH 4 and 7 had a trend of **1** > **U** > **P**, where the one carbon homolog again was the most stable. The corresponding quaternary ammonium series exhibited similar solution stability with trend of **O**<**A**<**T**. Cyclic ammonium compounds have better stability when they are substituted with EWG (**H**). We believe there are two possible reasons for this observation: 1) the EWGs increased the electropositive charge on the piperidinium nitrogen which in turn stabilizes the NCl_2; or 2) the EWGs possibly decrease the reactivity of the ring carbons to oxidation. Comparison of stabilities for **O**-**Q** at pH 4 indicates that dichloroamines had compatibility preference of NMe_3 > PMe_3 > SO_3H. The solution stability study suggested that stable compounds may have WSGs of sulfonic acid and quaternary ammonium with one methylene between the quaternary carbon and the WSG. In the case of a backbone extension a sulfone must have a two carbon spacer between the quaternary carbon and the WSG. Compound **R** is interesting because it has similar solution stability to **T** at pH 4 but at is very unstable pH 7. The instability may be due to the spacer between the nitrogen and sulfur being too short. Compound **L** also had an ethyl spacer and was also unstable at pH 4 and 7, however, compound **M** with a propyl spacer between nitrogen and sulfur is stable at both pHs. We believe the ethyl spacer is activated by both the ammonium group and the sulfonate in the presence of base to form a vinylsulfonic acid and a substituted dimethylamine. In the case of **L** the elimination of trimethylamine was observed during the synthesis of intermediate **8**.

In summary, we have described the synthesis and antibacterial activity of various analogs of 2-dichloramino-2-methylpropane-1-sulfonic acid sodium salt **1**. Diverse functional groups have been identified that provide stability to the molecules as well as groups that are tolerant to the *N*-chloramino functionality. These stable structural modifications allow tailoring of physiochemical properties of the dichloroamine series for use in new antimicrobial indications.

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