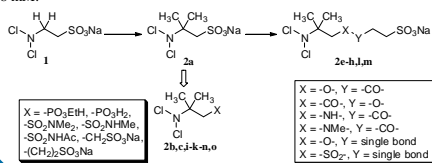
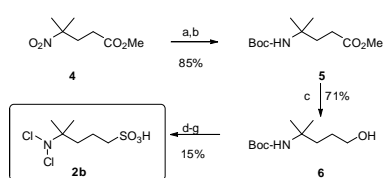


Abstract

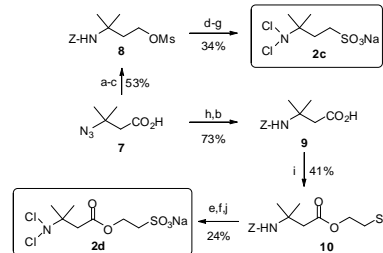
2-Dichloroamino-2-methylpropane-1-sulfonic acid sodium salt (**2a**), a stable derivative of endogenous *N,N*-dichlorotaurine (**1**), has been identified and is under development as a topical antimicrobial agent. Structure-activity relationships of analogs were explored to achieve optimal antimicrobial activity with minimal mammalian toxicity while maintaining the desired stability. All the analogs synthesized showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in the range of 4–40 μ M and cytotoxicity against mammalian L929 cells in the range 0.3–8 mM.



Homologated and Ester Analogs

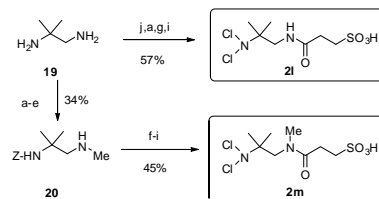


Scheme 1. Reagents and conditions: (a) AcOH, 10% Pd-C, H₂, 16h; (b) Boc₂O, CH₂Cl₂, Et₃N, 24h; (c) LiBH₄, THF, 0°C-25°C, 16h; (d) MeSO₂Cl, Et₃N, CH₂Cl₂, 0°C, 2h; (e) 4M-HCl/dioxane, 16h; (f) Aq. 1M Na₂SO₃, 25°C, 16h; (g) Aq. HOCl, 5°-10°C, 1h.



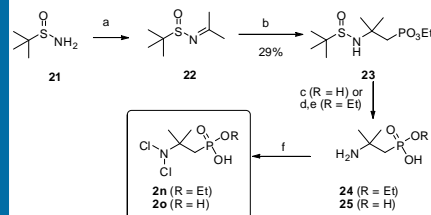
Scheme 2. Reagents and conditions: (a) LiAlH₄, ether, 0°C-25°C, 16h; (b) Z-OsU, isopropanol-H₂O, 16h; (c) MeSO₂Cl, Et₃N, CH₂Cl₂, 0°C, 2h (d) KSAC, DMF, 25°C, 16h; (e) HCO₂H, 30% H₂O₂, 25°C, 16h; (f) MeOH, 10% Pd-C, H₂, 12h, 25°C; (g) Aq. HOCl, 5°-10°C, 1h; (h) 10% Pd-C, H₂, HCO₂H, 12h, 25°C; (i) 3-(Acetylthio)ethanol, CDI, CH₂Cl₂, 70°C, 16h; (j) *tert*-Butyl hypochlorite, MeOH, 0°-25°C, 2h.

Reverse Amide Analogs



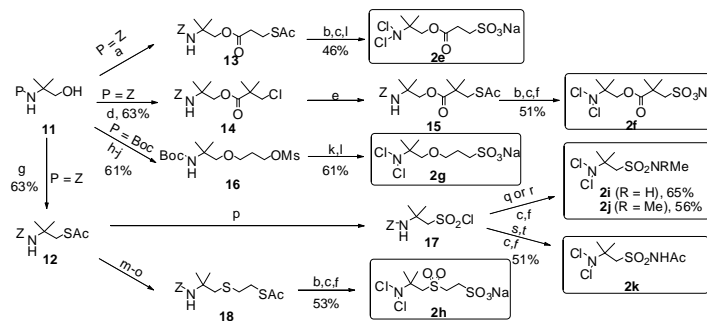
Scheme 4. Reagents and conditions: (a) Boc₂O, THF, -40°-25°C, 16h; (b) Z-OsU, isopropanol-H₂O, 16h; (c) 4M-HCl/dioxane, 25°C, 16h; (d) HCO₂H, CDI, DMF, 25°C; (e) BH₃Me₂, THF, 0°-25°C, 16h, MeOH-HCl; (f) 3-(Acetylthio)propanoic acid, CDI, DMF, 70°C, 16h; (g) HCO₂H, 30% H₂O₂, 16h; (h) MeOH, 10% Pd-C, H₂, 16h; (i) MeOH, *tert*-Butyl hypochlorite, 0°-25°C, 1h; (j) 3-(Acetylthio)propanoic acid, CDI, THF, -70°-25°C, 4h.

Phosphonate Analogs



Scheme 5. Reagents and conditions: (a) Acetone, Ti(OEt)₄, THF, reflux, 2 h (b) *n*-BuLi, TMEDA, THF, MePO₂Et₂, -78°C, 4h; (c) TMSBr, CH₂Cl₂, 65°C, 1h; (d) NaOH, EtOH-H₂O, 80°C, 12h; (e) MeOH, 4M-HCl/dioxane, 25°C, 1h; (f) Aq. HOCl, 5°-10°C, 1h.

Ether, Reverse Ester, Sulfone and Sulfonamide Analogs

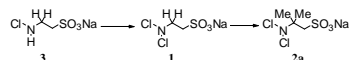


Scheme 3. Reagents and conditions: (a) 3-(Acetylthio)propanoic acid, CDI, DMF, 25°C, 16h; (b) HCO₂H, 30% H₂O₂, 25°C, 16h; (c) MeOH, 10% Pd-C, H₂, 25°C, 24h; (d) 3-Chloro-2,2-dimethylpropanoyl chloride, Et₃N, CH₂Cl₂, 25°C, 16h; (e) DMF, KSAC, 80°C, 3h; (f) *tert*-Butyl hypochlorite, MeOH, 0°-25°C, 2h; (g) AcSH, DIAD, PPh₃, THF, -5°-25°C (h) DMF, NaH, allyl bromide, 0°-25°C, 16h; (i) 9-BBN, THF, 25°C, 16h; (j) MeSO₂Cl, Et₃N, CH₂Cl₂, 0°C, 2h; (k) 4M-HCl/dioxane, 25°C, 16h, 1M aq. Na₂SO₃, 40°C, 16h; (l) Aq. HOCl, 5°-10°C, 1h; (m) MeOH-MeONa, 25°C, 4h; (n) 1-Bromo-2-chloroethane, Cs₂CO₃, DMF, 25°C, 16h; (o) KSAC, DMF, 70°C, 16h; (p) Aq. HOCl, CH₂Cl₂, 0°C, 0.5h; (q) 40% Aq. MeNH₂, 0°-25°C, 3h; (r) 40% Aq. Me₂NH, 0°-25°C, 3h; (s) 30% Aq. NH₃, 0°-25°C, 3h; (t) CH₂Cl₂, Ac₂O, DIPEA, 25°C, 16h.

Introduction

Bacteria have increasingly become resistant to most currently available antibiotics; hence, there is a continuing need for antimicrobial agents with novel mechanisms of action and low potential for the development of resistance. We embarked on a program for the development of *N*-chlorotaurine-based molecules as antimicrobial agents.

Taurine (2-aminoethanesulfonic acid) is a conditionally essential amino acid known to have various physiological functions.¹ *N*-chlorotaurine (**3**) and *N,N*-dichlorotaurine (**1**) are produced from taurine during the respiratory burst in activated neutrophils and in macrophages² via the scavenging of myeloperoxidase-produced hypochlorous acid.



Nagl³ *et al* have previously reported the bactericidal, fungicidal and virucidal activity of *N*-chlorotaurine (**3**). Due to their non-specific mechanism of action, this class of compounds has a low potential for the development of resistance. In spite of the antimicrobial activity and low cytotoxicity, therapeutic utility of *N*-chlorotaurine (**3**) is limited by its poor long-term solution stability at room temperature.⁴ We reasoned that *N,N*-dichlorotaurine (**1**) would be more stable but discovered that it still lacked the long-term stability required for a therapeutic agent. We presumed the transient nature of **1** to be due to rapid dehydrochlorination and introduced a dimethyl group at the β -carbon to block this transformation. Thus compound **2a** was identified⁵ as a stable analog of *N,N*-dichlorotaurine (**1**). To our surprise, **2a** exhibited a half life of >2 years at 40°C which gave us enough impetus to further develop this class of compounds.

We believed that the initial lead, **2a**, could be further optimized with respect to topical antimicrobial potency, *in vivo* efficacy and cytotoxicity by suitable structural modifications (Figure 1). We herewith report the design, synthesis and biological activity of various backbone modification and sulfonic acid replacement in **2a**.

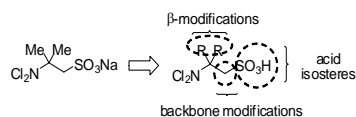


Figure 1. SAR strategy on *N,N*-dichlorodimethyltaurine **2a**.

Table 1. Biological activity of **2a** and its analogs

Compd	MBC or MFC (μ g/ml) ¹			CT ₅₀ (μ g/ml) mouse fibroblast L929 cells pH 4 (saline)
	<i>S. aureus</i> ATCC 29213 pH 4(saline)	<i>E. coli</i> ATCC 25922 pH 4(saline)	<i>C. albicans</i> ATCC 10231 pH 4(saline)	
2a	0.86 ^a	3.5 ^b	28 ^c	1200
2b	8 ^a	6.9 ^b	>128 ^c	640
2c	4	4 ^b	16	1900
2d	2	2	64	260
2e	1 ^a	2	4.0 ^c	130
2f	8	4	16	840
2h	8	4	64	94
2k	0.5	4	8	ND
2n	2 ^c	8 ^b	32 ^c	80
2o	16	8	16	130

¹MBC 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 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.

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 4 μ g/ml in the case of compound **2e** to greater than 128 μ g/ml in the case of compound **2b**. In terms of cytotoxicity, the phosphonate analogs, **2n** and **2o**, as well as the reverse ester **2e** had the highest toxicity, about 10-fold higher than the lead compound **2a**, but all compounds had therapeutic indices (ratio of CT₅₀ to MBC) between 10 and 1900 for bacteria and between 2 and 40 for *C. albicans*. Since the antimicrobial activity of these molecules is due to the oxidative capacity of the *N*-chloroamine functionality, we did not observe any significant SAR among the analogs.

In summary, we have described the synthesis and antibacterial activity of various analogs of 2-dichloroamino-2-methylpropane-1-sulfonic acid sodium salt **2a**. Diverse functional groups have been identified that provide stability to the molecules as well as groups that are tolerant to the *N*-chloroamine functionality. These molecules have been evaluated as backups for our lead clinical candidate, compound **2a**.

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