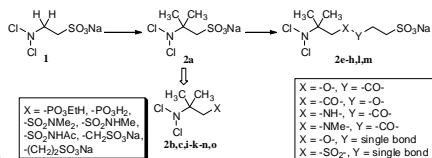
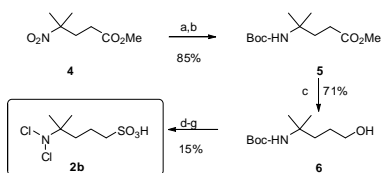


## Abstract

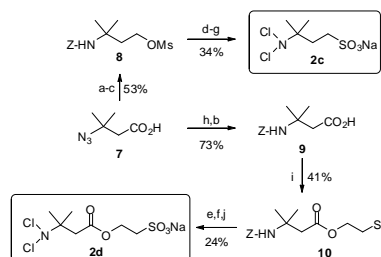
2-Dichloroamino-2-methyl-propane-1-sulfonic acid sodium salt (**2a**), a stable derivative of endogenous *N,N*-dichlorotaurine (**1**), has been identified and is under development as a topical antimicrobial agent. Structure-activity relationships of analogs were explored to achieve optimal antimicrobial 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  $\mu$ M and cytotoxicity against mammalian L929 cells in the range 0.3-8 mM.



## Homologated and Ester Analogs

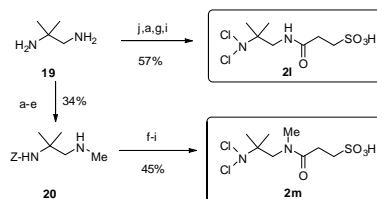


Scheme 1. Reagents and conditions: (a) AcOH, 10% Pd-C, H<sub>2</sub>, 16h; (b) Boc<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>, Et<sub>3</sub>N, 24h; (c) LiBH<sub>4</sub>, THF, 0°C-25°C, 16h; (d) MeSO<sub>2</sub>Cl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0°C, 2h; (e) 4M-HCl/dioxane, 16h; (f) Aq. 1M Na<sub>2</sub>SO<sub>3</sub>, 25°C, 16h; (g) Aq. HOCl, 5°-10°C, 1h.



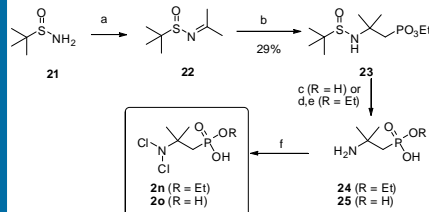
Scheme 2. Reagents and conditions: (a) LiAlH<sub>4</sub>, ether, 0°C-25°C, 16h; (b) Z-OSu, isopropanol-H<sub>2</sub>O, 16h; (c) MeSO<sub>2</sub>Cl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0°C, 2h (d) KSAc, DMF, 25°C, 16h; (e) HCO<sub>2</sub>H, 30% H<sub>2</sub>O<sub>2</sub>, 25°C, 16h; (f) MeOH, 10% Pd-C, H<sub>2</sub>, 12h, 25°C; (g) Aq. HOCl, 5°-10°C, 1h; (h) 10% Pd-C, H<sub>2</sub>, HCO<sub>2</sub>H, 12h, 25°C; (i) 3-(Acetylthio)ethanol, CDI, CH<sub>2</sub>Cl<sub>2</sub>, 70°C, 16h; (j) *tert*-Butyl hypochlorite, MeOH, 0°-25°C, 2h.

## Reverse Amide Analogs



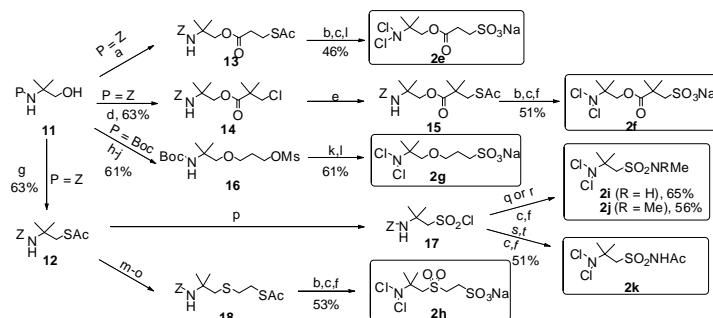
Scheme 4. Reagents and conditions: (a) Boc<sub>2</sub>O, THF, -40°-25°C, 16h; (b) Z-OSu, isopropanol-H<sub>2</sub>O, 16h.; (c) 4M-HCl/dioxane, 25°C, 16h; (d) HCO<sub>2</sub>H, CDI, DMF, 25°C; (e) BH<sub>3</sub>Me<sub>2</sub>S, THF, 0°-25°C, 16h, MeOH-HCl; (f) 3-(Acetylthio)propanoic acid, CDI, DMF, 70°C, 16h; (g) HCO<sub>2</sub>H, 30% H<sub>2</sub>O<sub>2</sub>, 16h; (h) MeOH, 10% Pd-C, H<sub>2</sub>, 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)<sub>4</sub>, THF, reflux, 2 h (b) *n*-BuLi, TMEDA, THF, MePO<sub>2</sub>Et<sub>2</sub>, -78°C, 4h; (c) TMSBr, CH<sub>3</sub>CN, 65°C, 1h; (d) NaOH, EtOH-H<sub>2</sub>O, 80°C, 12h; (e) MeOH, 4M-HCl/dioxane, 25°C, 1h; (f) Aq. HOCl, 5°-10°C, 1h.

## Ether, Reverse Ester, Sulfone and Sulfonylamide Analogs

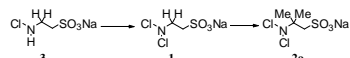


Scheme 3. Reagents and conditions: (a) 3-(Acetylthio)propanoic acid, CDI, DMF, 25°C, 16h; (b) HCO<sub>2</sub>H, 30% H<sub>2</sub>O<sub>2</sub>, 25°C, 16h; (c) MeOH, 10% Pd-C, H<sub>2</sub>, 25°C, 24h; (d) 3-Chloro-2,2-dimethylpropanoyl chloride, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 25°C, 16h; (e) DMF, KSAc, 80°C, 3h; (f) *tert*-Butyl hypochlorite, MeOH, 0°-25°C, 2h.; (g) AcSH, DIAD, PPh<sub>3</sub>, THF, -5°-25°C (h) DMF, NaH, allyl bromide, 0°-25°C, 16h; (i) 9-BBN, THF, 25°C, 16h; (j) MeSO<sub>2</sub>Cl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0°C, 2h; (k) 4M-HCl/dioxane, 25°C, 16h.; (l) 1M aq. Na<sub>2</sub>SO<sub>3</sub>, 40°C, 16h; (m) Aq. HOCl, 5°-10°C, 1h; (n) MeOH-MeONa, 25°C, 4h; (o) 1-Bromo-2-chloroethane, Cs<sub>2</sub>CO<sub>3</sub>, DMF, 25°C, 16h; (p) KSAc, DMF, 70°C, 16h; (q) Aq. HOCl, CH<sub>2</sub>Cl<sub>2</sub>, 0°C, 0.5h; (r) 40% Aq. MeNH<sub>2</sub>, 0°-25°C, 3h; (s) 40% Aq. Me<sub>2</sub>NH, 0°-25°C, 3h; (t) CH<sub>2</sub>Cl<sub>2</sub>, Ac<sub>2</sub>O, 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.<sup>1</sup> *N*-chlorotaurine (**3**) and *N,N*-dichlorotaurine (**1**) are produced from taurine during the respiratory burst in activated neutrophils and in macrophages<sup>2</sup> via the scavenging of myeloperoxidase-produced hypochlorous acid.



Nagl<sup>3</sup> *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.<sup>4</sup> 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  $\beta$ -carbon to block this transformation. Thus compound **2a** was identified<sup>5</sup> 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**.

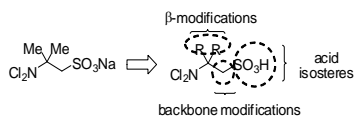


Figure 1. SAR strategy on *N,N*-dichlorodimethyltaurine **2a**.

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

Compd	MBC or MFC ( $\mu$ g/ml) <sup>1</sup>			CT <sub>50</sub> ( $\mu$ 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)	
<b>2a</b>	0.86 <sup>a</sup>	3.5 <sup>b</sup>	28 <sup>c</sup>	1200
<b>2b</b>	8 <sup>a</sup>	6.9 <sup>b</sup>	>128 <sup>c</sup>	640
<b>2c</b>	4	4 <sup>b</sup>	16	1900
<b>2d</b>	2	2	6.0	260
<b>2e</b>	1 <sup>a</sup>	2	4.0 <sup>f</sup>	130
<b>2f</b>	8	4	16	840
<b>2h</b>	8	4	64	94
<b>2k</b>	0.5	4	8	ND
<b>2n</b>	2 <sup>a</sup>	8 <sup>b</sup>	32 <sup>c</sup>	80
<b>2o</b>	16	8	16	130

<sup>1</sup>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 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  $\mu$ g/ml in the case of compound **2e** to greater than 128  $\mu$ 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 CT<sub>50</sub> 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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