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N,N-Dichloroaminosulfonic acids as novel topical antimicrobial agents

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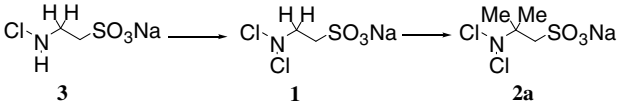
ABSTRACT

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 1–128 μg/mL and cytotoxicity against mammalian L929 cells in the range 80–1900 μg/mL.

Bacteria are increasingly resistant to most currently available antibiotics. To meet the continuing need for antimicrobial agents with novel mechanisms of action and low potential for development of resistance we initiated a program for the development of *N*-chlorotaurine-based molecules as topical 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 macrophages[2] via the scavenging of myeloperoxidase-produced hypochlorous acid.



3 **1** **2a**

Nagl et al.[3] previously described the bactericidal, fungicidal and virucidal activity of *N*-chlorotaurine. Due to their non-specific mechanism of action, this class of compounds has low potential for the development of resistance. Despite potent antimicrobial activity and low cytotoxicity, therapeutic use of *N*-chlorotaurine is limited by its poor long-term solution stability at room temperature.[4] We believed *N,N*-dichlorotaurine (**1**) would be more stable but discovered it still lacked the long-term stability required for therapeutic agents. We presumed the transient nature of **1** was due to rapid dehydrochlorination and introduced a dimethyl group at the β-carbon to block this transformation. Thus compound **2a** was identified[5] as a stable analog of **1**, exhibiting a half life of >2 years at 40 °C and provided impetus to further develop this class of compounds.

We expected that our initial lead, **2a**, could be further optimized for topical antimicrobial potency, in vivo efficacy and cytotoxicity by suitable structural modifications (Fig. 1). Here, we report the design, synthesis, and biological activity of various backbone modification and sulfonic acid replacements in **2a**.

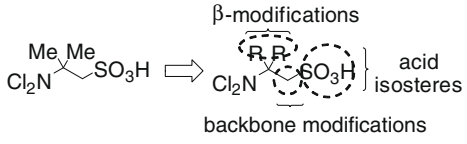
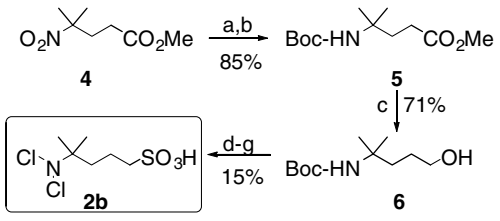


Figure 1. SAR strategy on *N,N*-dichlorotaurine **2a**.



Scheme 1. Reagents and conditions: (a) AcOH, 10% Pd–C, H_2, 16 h; (b) Boc_2O, CH_2Cl_2, Et_3N, 24 h; (c) $LiBH_4$, THF, 0–25 °C, 16 h; (d) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 2 h; (e) 4 M HCl/dioxane, 16 h; (f) aq 1 M Na_2SO_3, 25 °C, 16 h; (g) aq HOCl, 5–10 °C, 1 h.

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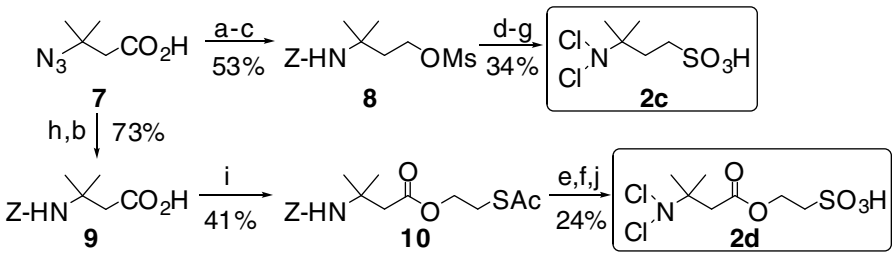
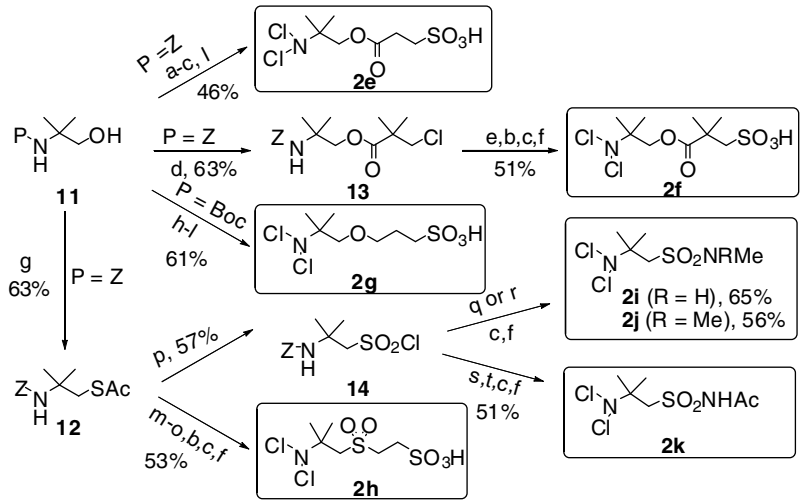


Scheme 2. Reagents and conditions: (a) $LiAlH_4$, ether, 0–25 °C, 16 h; (b) *Z*-OSu, isopropanol–H_2O, 16 h; (c) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 2 h; (d) KSAc, DMF, 25 °C, 16 h; (e) HCO_2H, 30% H_2O_2, 25 °C, 16 h; (f) MeOH, 10% Pd–C, H_2, 12 h, 25 °C; (g) aq HOCl, 5–10 °C, 1 h; (h) 10% Pd–C, H_2, HCO_2H, 12 h, 25 °C; (i) 2-(Acetylthio)ethanol, CDI, CH_3CN, 70 °C, 16 h; (j) *tert*-Butyl hypochlorite, MeOH, 0–25 °C, 2 h.



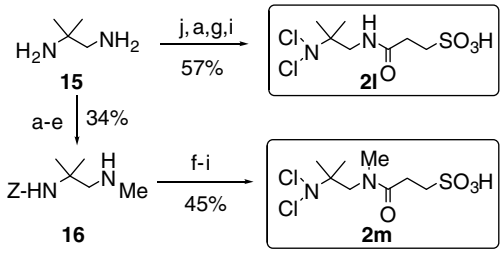
Scheme 3. Reagents and conditions: (a) 3-(Acetylthio)propanoic acid, CDI, DMF, 25 °C, 16 h; (b) HCO_2H, 30% H_2O_2, 25 °C, 16 h; (c) MeOH, 10% Pd–C, H_2, 25 °C, 24 h; (d) 3-Chloro-2,2-dimethylpropanoyl chloride, Et_3N, CH_2Cl_2, 25 °C, 16 h; (e) DMF, KSAc, 80 °C, 3 h; (f) *tert*-Butyl hypochlorite, MeOH, 0–25 °C, 2 h; (g) AcSH, DIAD, PPh_3, THF, −5 to 25 °C; (h) DMF, NaH, allyl bromide, 0–25 °C, 16 h; (i) 9-BBN, THF, 25 °C,16 h; (j) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 2 h; (k) 4 M HCl/dioxane, 25 °C, 16 h, aq 1 M Na_2SO_3, 40 °C, 16 h; (l) aq HOCl, 5–10 °C, 1 h; (m) MeOH–MeONa, 25 °C, 4 h; (n) 1-bromo-2-chloroethane, Cs_2CO_3, DMF, 25 °C, 16 h; (o) KSAc, DMF, 70 °C, 16 h; (p) HOCl, H_2O, CH_2Cl_2, 0.5 h; (q) 40% aq $MeNH_2$, 5–25 °C, 3 h; (r) 40% aq Me_2NH, 0–25 °C, 3 h; (s) 30% aq NH_3, 5–25 °C, 3 h; (t) CH_2Cl_2, Ac_2O, DIPEA, 25 °C, 16 h.

Our first course of action was to synthesize analogs with β-modifications. This series of analogs were surprisingly unstable and was presented elsewhere.[6]

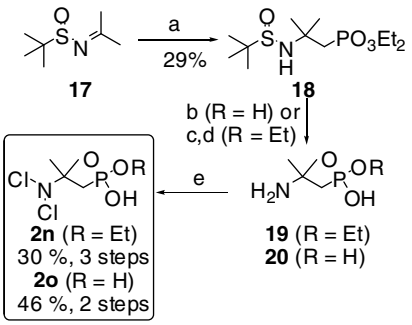
The synthesis of *N,N*-dichloroamine **2b** began with the key intermediate **5**, prepared from methyl 4-methyl-4-nitropentanoate **4** as shown in Scheme 1. The alcohol in **6** was converted into the sulfonic acid group in two steps, through the mesylate intermediate which was displaced with Na_2SO_3 to yield the sulfonic acid. The final chlorination was achieved with hypochlorous acid (formed in situ from sodium hypochlorite under acidic pH) to furnish the *N,N*-dichloro compound **2b**.

The chloramine **2c** was synthesized from azide **7**[7] following the sequence of steps illustrated in Scheme 2. LAH reduced both the acid and the azido functionality in **7** to the amino alcohol, which was *Z*-protected and converted to the mesylate **8**. Reaction with sodium sulfite and chlorination as described above afforded **2c**.

We also introduced various functional groups in the backbone to gain an understanding of the tolerance of these groups to the



Scheme 4. Reagents and conditions: (a) Boc_2O, THF, −40 to 25 °C, 16 h; (b) *Z*-OSu, isopropanol–H_2O, 16 h, (c) 4 M HCl/dioxane, 25 °C, 16 h; (d) HCO_2H, CDI, DMF, 25 °C; (e) $BH_3{\cdot}Me_2S$, THF, 0–25 °C, 16 h, 1 M MeOH–HCl; (f) 3-(Acetylthio)propanoic acid, CDI, DMF, 70 °C, 16 h; (g) HCO_2H, 30% H_2O_2, 16 h; (h) MeOH, 10% Pd–C, H_2, 16 h; (i) MeOH, *tert*-Butyl hypochlorite, 25 °C, 1 h; (j) 3-(Acetylthio)propanoic acid, CDI, THF, −70 °C to rt, 4 h.



Scheme 5. Reagents and conditions: (a) *n*-BuLi, TMEDA, THF, $MePO_3Et_2$, −78 °C, 4 h; (b) TMSBr, CH_3CN, 65 °C, 1 h; (c) NaOH, EtOH–H_2O, 80 °C, 12 h; (d) MeOH, 4 M HCl/dioxane, 25 °C, 1 h; (e) aq HOCl, 5–10 °C, 1 h.

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

Compound	MBC or MFC[a] (μg/mL)			CT_{50} (μg/mL) Mouse fibroblast L929 cells pH 4 (saline)	$t_{½}$ pH 4 (saline) (days)
	S. aureus ATCC 29213	*E. coli* ATCC 25922	*C. albicans* ATCC 10231		
2a	1[b]	4[c]	32[d]	1200	>200
2b	8[b]	8[c]	>128[d]	640	16
2c	4	4	16	1900	174
2d	2	2	64	270	>95
2e	1[b]	2	4[d]	130	64
2f	8	4	16	840	80
2h	8	4	64	1820	>95
2k	1	2	64	ND	44
2n	2[b]	ND	32[d]	80	>18
2o	16	8	16	130	23

[a] MBC is determined using a modification of a standard method described in CLSI M26-A where Mueller–Hinton broth (MHB) is replaced by isotonic saline at pH 4 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 to 20 h at 35 °C to 1 h at room temperature.
[b] *S. aureus* MCC 91731.
[c] *E. coli* MCC 80392.
[d] *C. albicans* MCC 50319.

chloramino functionality. The ester analog **2d** (Scheme 2) was prepared from the acid **9**, which was obtained by the hydrogenation of azide **7** using Pd–C in formic acid. Coupling of **9** with *S*-2-hydroxyethyl ethanethiolate under CDI[8] coupling conditions gave the intermediate thioacetate **10**. Oxidation of **10**, followed by N-deprotection and chlorination using *tert*-butyl hypochlorite afforded the final dichloramine **2d**.

We next introduced a range of functional groups in the backbone of **2a** as depicted in Scheme 3. The protected amino alcohol **11** and its thioacetate derivative **12** served as common starting materials for most of these analogs. Thus, the coupling of the amino alcohol **11** with 3-(acetylthio)propionic acid[9] under CDI conditions afforded an intermediate thioacetate, which was oxidized to sulfonic acid using HCO_2H–H_2O_2. N-Chlorination using HOCl as before yielded the desired reverse ester analog **2e**. The sterically hindered ester **2f** was also synthesized from **11** and 3-chloro-2,2-dimethylpropanoyl chloride. The coupled ester **13** on reaction with potassium thioacetate followed by oxidation gave the sulfonic acid. N-Deprotection and chlorination using *tert*-butyl hypochlorite yielded the *N,N*-dichloro analog **2f** (Scheme 3).

The ether-linked and sulfone-linked analogs **2g** and **2h**, respectively, were also synthesized (Scheme 3). The ether analog **2g** was synthesized from **11** following reaction steps as shown in Scheme 3. Allylation of **11** with allyl bromide followed by hydroboration, mesylation, displacement with sodium sulfite, and chlorination with HOCl afforded **2g**. The sulfone **2h** was accessed from thioacetate **12**, obtained by a direct Mitsunobu reaction[10] between the alcohol **11** and thioacetic acid followed by reaction sequences shown in Scheme 3.

Several sulfonic acid replacements, like sulfonamide and *N*-acetyl sulfonamide analogs, were also synthesized from thioacetate intermediate **12**. Treatment of **12** with hypochlorous acid led to the formation of the corresponding sulfonyl chloride **14**, which was reacted with methylamine or dimethylamine to afford **2i** and **2j**, respectively after N-deprotection and chlorination using *tert*-butyl hypochlorite. Similarly, the use of ammonia provided the primary sulfonamide, which was treated with acetic anhydride followed by N-deprotection and chlorination as described for the preparation of **2i** and **2j**, gave **2k**.

Amide-linked analogs **2l** and **2m** were prepared from commercially available building block **15** (Scheme 4). Coupling of **15** with 3-(acetylthio)propanoic acid under CDI conditions followed by oxidation and N-chlorination as described earlier yielded compound **2l**. Similarly, compound **2m** was synthesized from the monoprotected diamine **16** in four steps as shown in Scheme 4. The sulfonic acid group of compound **2a** was also replaced with acid isosteres such as phosphonates. Phosphonate analogs **2n** and **2o** were prepared from the sulfinimine **17**[11] as illustrated in Scheme 5. Addition of ((diethoxyphosphoryl)methyl) lithium to **17** provided **18**, which on selective hydrolytic conditions (Scheme 5) gave **19** or **20**. Chlorination using HOCl provided the desired dichloramines **2n** and **2o**.

The data in Table 1 summarizes 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 *Candida albicans* was the most variable for the compounds tested, ranging from 4 ug/mL in the case of compound **2e** to greater than 128 μg/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 in vitro toxicity, about 10-fold higher than the lead compound **2a**; however all compounds had therapeutic indices (ratio of CT_{50} to MBC) from 8 to 1200 for bacteria and from 2 to 118 for *C. albicans*. Since the antimicrobial activity of these molecules is due to the oxidative capacity of the dichloramine functionality, we did not observe any significant SAR among the analogs in this class.

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

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