MEDI 311

Towards the development of shelf-stable *N*-chloroamines as topical anti-microbial agents

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American Chemical Society National Meeting, Denver, CO, Aug 28th – Sept 1st, 2011

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Abstract

N-Chloroamines are potent, fast-acting antimicrobial agents. However, organic *N*-chloroamines have not been commercialized as well-tolerated topical anti-microbial agents for human or animal use. NVC-422 is a stable 2,2-dimethyl analog of the endogenous antimicrobial *N,N*-dichlorotaurine with more than a 2 year shelf-life at room temperature. NVC-422 is being evaluated in Phase II clinical trials for impetigo and viral conjunctivitis. Recent investigations on developing additional *N*-chloroamines, beyond NVC-422, show subtleties in the substitution pattern can influence the physicochemical properties and the overall stability of these newer compounds. We describe the influence of the stereoelectronic environment around the chloramine nitrogen and the overall lipophilicity of the molecule on the stability of new analogs in preliminary formulations.

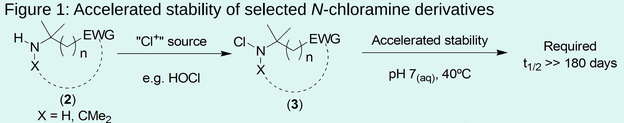
Background & Introduction

N-Chlorotaurine (NCT) and *N,N*-dichlorotaurine (NNDCT) are produced by hypochlorous acid chlorination of taurine following oxidative burst in activated neutrophils. These molecules are very effective, natural antimicrobials, and form an important part of the innate immune system's response to infection.[1] The chemical and bactericidal properties of *N,N*-dichlorotaurine have been discussed.[2] NVC-422 (**1**), is a stable 2,2-dimethyl analog of endogenous antimicrobial NNDCT that exhibits good long-term aqueous solution stability,[3] shows suitability for topical anti-infective applications and has recently been evaluated in clinical trials for treatment of impetigo and viral conjunctivitis. Recent investigations on analogues of (**1**) revealed that subtleties in the substitution pattern have significant influences on stability of the parent *N*-chloro bond in aqueous media (Figure 1), and ultimately on the shelf-life of the final therapeutic agent.[3b,4] These materials are increasingly stable, and display improved antimicrobial activity, at lower pH.

Search for factors influencing stability

Herein we explore the importance of the stereoelectronic environment around the nitrogen atom and the overall lipophilicity of the molecule as we tried to identify windows of stability during our continuing efforts to identify antimicrobial and virucidal agents with improved potency and spectrum. To identify the key parameters determining aqueous stability, particularly at neutral pH, we examined a series (**3**) of acyclic *N,N*-dichloroamines and cyclic *N*-chloroamines, varying the nature of the stabilizing electron withdrawing group (EWG), distance of the EWG from the *N*-chloro bond, charge at neutral pH, and lipophilicity of the final *N*-chloro derivative. We used the calculated pKa of the starting amine (**2**) as a surrogate to quantify the influence of the EWG, and cLogD or ChromLogD as a measure of lipophilicity.

Figure 1: Accelerated stability of selected *N*-chloramine derivatives



(**2**) X = H, CMe_2 (**3**)

(**1**) $t_{1/2}$ = 302 days; (**4**) $t_{1/2}$ = 110 days[3b]; (**5**) $t_{1/2}$ = 1 day[4a]; (**6**) $t_{1/2}$ > 200 days[4c]; (**7**) $t_{1/2}$ > 396 days[2]

Materials & Methods

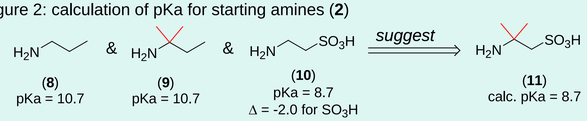
Synthesis of the *N*-chloroamine and *N,N*-dichloroamine derivatives reported here have already been described.[3,4]

Accelerated aging studies were conducted at 40 ºC in the dark, as 5 mM aqueous solutions containing 0.9% NaCl, buffered with either 20 mM phosphate (pH 7) or 5 mM Acetate (pH 4). Aliquots were analyzed by HPLC at either 304 nm (for *N,N*-dichloroamines: λmax ~ 308 nm) or 254 nm (for *N*-monochloroamines: λmax = 248 - 280 nm).

The pKa for protonation of the starting amine derivatives, e.g. (**11**) was estimated by comparison with the pKa of known molecular fragments (Figure 2).[5]

There are several programs that can calculate LogD values; however, not all can accommodate the N-Cl functionality. Uncorrected LogD values at pH 7.4 (cLogD7.4) were determined using the structure drawing program Marvin Sketch from Chem Axon. Experimental determination of LogD at pH 7.4 (ChromLog$D_{7.4}$) was accomplished by reverse-phase HPLC on a Waters Symmetry C8 column, and comparing the retention time to known standards, according to the method of Valko.[6]

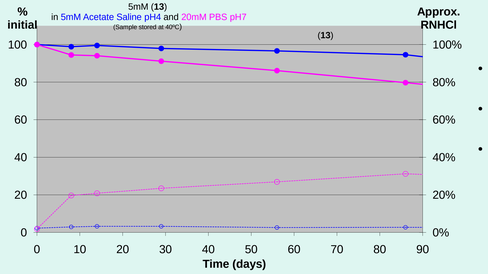
Figure 2: calculation of pKa for starting amines (**2**)



(**8**) pKa = 10.7; (**9**) pKa = 10.7; (**10**) pKa = 8.7, Δ = -2.0 for SO_3H; (**11**) calc. pKa = 8.7

Results

Figure 3: Percent of (**13**) remaining at pH 4 (solid blue), pH 7 (solid magenta), percent monochloro at pH 4 (dashed blue) and pH 7 (dashed magenta)



- Stability was tracked by measuring the percent remaining by HPLC, NMR, or UV, and expressed as $t_{1/2,}$ the number of days for the solution to reach 50% remaining. See Tables 1 and 2.
- In cases where compounds are exceptionally stable, the $t_{1/2}$ was estimated from the data available using an exponential decay model.
- The corresponding monochloro derivatives were also tracked by HPLC and constitute the major decomposition product at pH 7. Further studies on the fate of the chlorine are underway.

Figure 4: Stability of *N,N*-dichloroamines (Table 1) vs. pKa of corresponding amine

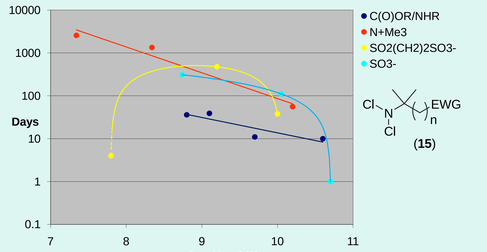


Figure 5: Stability of *N*-chloroamines (Table 2) vs. pKa of corresponding amine

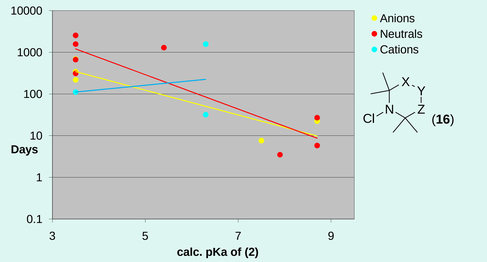
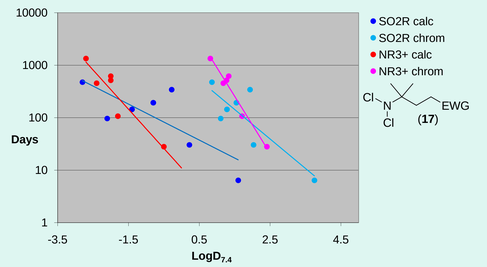


Figure 6: Stability of N-chloroamines (Table 1) vs. lipophilicity (Log$D_{7.4}$)



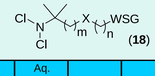


Table 1: Stability and property values for *N,N*-dichloramines

CMPD #	$(CH_2)_m$	"X" - EWG	$(CH_2)_n$	WSG	Charge @ pH 7.4	Estimated pKa of SM amine	Aq. Stability @ pH 4 40 ºC t½ (days)	Aq. Stability @ pH 7 40 ºC t½ (days)	Chrom $LogD_{7.4}$	$cLogD_{7.4}$
19	1	C(=O)NHR	2	SO_3^-	-1	8.8	33	36	-	-2.6
20	1	C(=O)OR	2	SO_3^-	-1	9.1	350*	39	-	-1.9
21	2	C(=O)OMe			0	9.7	52	11	-	1.8
22	2	CO_2H			-1	10.6	17	10	-	-1
23	1	N^+Me_3			1	7.3	2200*	2600*	0.3	-2.8
7	2	N^+Me_3			1	8.3	1870*	1340*	0.8	-2.7
24	3	N^+Me_3			1	10.2	117*	56	1.1	-2.3
25	2	N^+MeEt_5			1	8.3	>1240*	455*	1.2	-2.4
26	2	$N^+Me(CH_2)_5$			1	8.3	620*	520*	1.3	-2
27	2	$N^+Me(4,4\text{-}F_2)c(CH_2)_5$			1	8.3	>1170*	620*	1.3	-2
28	2	N^+Me_2Bu			1	8.3	>107*	107	1.7	-1.8
29	2	N^+Me_2Hex			1	8.3	207*	28	2.4	-0.5
30	3	P^+Me_3			1	10.7	46	26	-	3.3
1	1	SO_3^-			-1	8.7	1210*	310*	-	-1.7
4	2	SO_3^-			-1	10.1	174	111	-	-1.6
31	3	SO_3^-			-1	10.7	6	1	-	-1.2
32	2	SO_3NRMe	2	OH	0	9.2	630*	170	1.8	-0.76
33	2	SO_3NRMe	3	OH	0	9.2	1540*	226	2.0	-
12	1	SO_2	2	SO_3^-	-1	7.8	193*	4	-	-2.9
13	2	SO_2	2	SO_3^-	-1	9.2	1260*	475*	0.9	-2.8
14	3	SO_2	2	SO_3^-	-1	10	47	38	-	-3
34	2	SO_2	1	$(CHOH)_2$	0	9.2	900*	144	1.3	-1.4
35	2	SO_2	2	OH	0	9.2	870*	193*	1.5	-0.8
36	2	SO_2	3	OH	0	9.2	590*	96*	1.1	-2.1

* Half life extrapolated

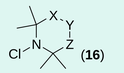


Table 2:Stability and property values for cyclic *N*-chloroamines

CMPD #	X	Y	Z	Charge @ pH 7.4	Estimated pKa of SM amine	Aq. Stability @ pH 4 40 ºC t½ (days)	Aq. Stability @ pH 7 40 ºC t½ (days)	Chrom $LogD_{7.4}$	$cLogD_{7.4}$
37	-	$N(CH_2)_3SO_3^-$	C=O	-1	3.5	880*	345*	-	-2.6
38	-	$N(CH_2)_4SO_3^-$	C=O	-1	3.5	665*	700*	-	-2.1
39	-	$N(CH_2)_5SO_3^-$	C=O	-1	3.5	1160*	218	-	-
40	CH_2	$C(OH)(CH_2)_2SO_3^-$	CH_2	-1	8.7	12	22.4	-	-
41	C=O	$N(CH_2)_3SO_3^-$	C=O	-1	7.5	7.6	7.6	-	-2.5
42	-	redacted	C=O	0	3.5	334*	310*	2.0	0.6
43	-	redacted	C=O	0	3.5	950*	670*	2.0	0.7
44	-	$N(CH_2)_2OH$	C=O	0	3.5	2550*	1570*	1.3	-
45	-	$N(CH_2)_4OH$	C=O	0	3.5	2550*	2550*	-	-
6	CH_2	SO_2	CH_2	0	5.4	1200*	1300*	2.3	0.2
46	CH_2	C=O	CH_2	0	7.9	4	3.5	-	1.6
47	CH_2	CH(OH)	CH_2	0	8.7	32	27	1.9	0.9
48	CH_2	CMe(OH)	CH_2	0	8.7	5.1	5.8	2.5	-
49	-	$N(CH_2)_3NMe_3^+$	C=O	1	3.5	216	111	-	-3.4
50	CH_2	CHN+Me_3	CH_2	1	6.3	28	32	-	-2.6
51	CH_2	$CHN^+Me_2(CH_2)_2OH$	CH_2	1	6.3	190*	1570*	-	-2.1

* Half life extrapolated

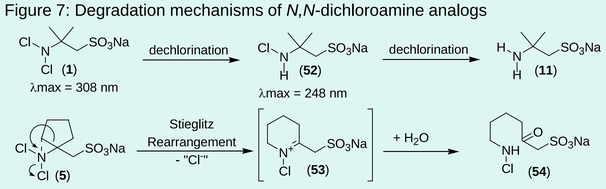
Discussion

Although historic *N*-chloramine derivatives are effective topical antimicrobial agents, their therapeutic deployment has been compromised by poor stability in aqueous formulations.[1,2,3] While developing our pipeline of Aganocide® compounds as topical antimicrobials, we found that aqueous solution stability was somewhat challenging to predict with these unique agents.

Generating a stability profile at 40 ºC is used for targeting a potential shelf-life of > 2 years at storage temperatures ≤ 25 ºC. The agents show improved shelf-life in aqueous solution at higher concentrations, and hence are deployed in concentrations ranging from 0.1% - 2% depending on the innate solubility of the analogs being evaluated (data not shown).

Multiple analogs have been identified that have predicted shelf lives > 2 years, either as anionic (**1**), (**13**), netural (**6**), (**44**), or cationic examples (**7**), (**23**) (Tables 1 & 2). *N,N*-dichloramines are more stable at lower pH, and more mono-chloroamine is produced at pH 7 vs. pH 4 (Figure 3). A parabolic dependence on amine pKa is observed for anionic N,N-dichloroamines (**1**), (**4**), (**31**) and (**12**), (**13**), (**14**), suggesting an optimal electronic character for the N-Cl bond (Figure 4). However, this parabolic dependence is not apparent for the cationic anlogs (**7**), (**23**) and (**24**). Again, a generalized trend of increasing stability with decreasing amine pKa is observed for cyclic *N*-chloramines (Figure 5). Finally, lipophilicity shows a correlation with both cLog$D_{7.4}$ and ChromLog$D_{7.4}$ for quaternary ammonium stabilized analogs (**7**), (**25**) – (**29**), with somewhat more scatter seen in the data for sulfonyl stabilized examples (**13**), (**32**) - (36) (Figure 6).

Figure 7: Degradation mechanisms of *N,N*-dichloroamine analogs



Conclusions

- ❖ *N,N*-Dichloroamines and cyclic *N*-chloroamines show good stability profiles at lower pH.
- ❖ The pKa of the precursor amine may be helpful as a surrogate marker for predicting a trend for the electronic character of the N-Cl bond until better molecular modeling programs can be employed.
- ❖ The overall lipophilicity of the agents influences the aqueous stability and therefore warrants further investigation.

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Acknowledgements

We would like to thank Drs. John Bartell, Howard Ketelson, Masood Chowhan, and David Stroman for helpful discussions. This work was supported by Alcon Laboratories, Inc.