Poster # 16423

Water-soluble *N*-chloroheterocycles as topical antimicrobial agents

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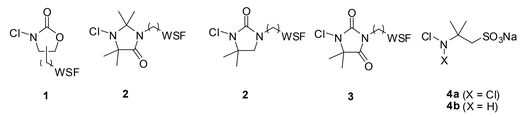
Abstract

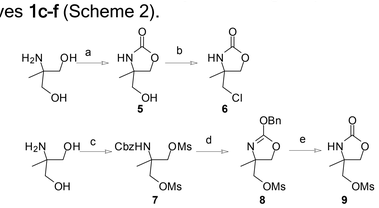
N-Chloroamines [1] are potent antimicrobial agents with excellent activity at lower pH (pH 3-5). For applications in which physiological pH is required, compounds with pH-independent activity are desired. Water-soluble *N*-chlorooxazolidinones (**1**), *N*-chloroimidazolidinones (**2**) and *N*-chlorohydantoins (**3**) were synthesized and their antimicrobial activities assayed at pHs 4 and 7. All compounds are active against a diverse panel of Gram-positive bacteria (*S. aureus*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *S. marcesens*), and yeast (*C. albicans*). Like *N*-chloroamines (**4**), *N*-chlorinated heterocycles show good selectivity for microbes over human cells (therapeutic indices of 0.2-100). However, unlike *N*-chloroamines, *N*-chlorinated heterocycles showed 0.5-32 fold better activity at pH 7 compared to pH 4. WSF = water solubilizing functionality (SO_3^-, NMe_3^+, etc.).



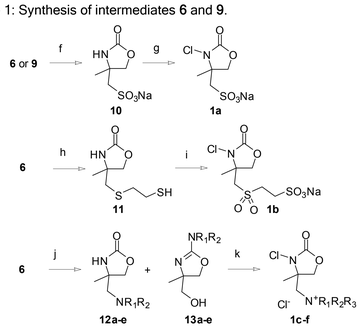
1 **2** **2** **3** **4a** (X = Cl) **4b** (X = H)

4,4-Disubstituted Oxazolidinones

3-Chloro-oxazolidin-2-ones **1a-f** were synthesized from a chloride (**6**) or mesylate (**9**) intermediate, made from 2-amino-2-methyl-1,3-propanediol as shown in Scheme 1. The intermediate was reacted with either a sulfur-based nucleophile to give the sodium sulfonate derivatives **1a-b**, or a secondary amine to give quaternary ammonium derivatives **1c-f** (Scheme 2).



Scheme 1: Synthesis of intermediates **6** and **9**.



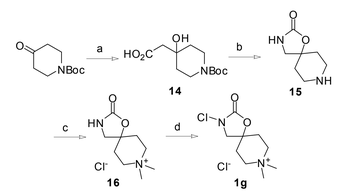
Scheme 2: Completion of 3-chlorooxazolidinones **1a-f**.

Conditions: (a) $(EtO)_2CO$, 150°C, (b) $SOCl_2$, 80°C, (c) CbzOSu, then MsCl, pyridine, 0°C, (d) NEt_3, 7 d, (e) H_2, Pd/C, (f) Na_2SO_3, DMF/H_2O, 50°C, (g) ^{t}BuOCl, 0°C, (h) EDT, 80°C, (i) H_2O_2, HCO_2H then ^{t}BuOCl, 0°C, (j) HNR_1R_2, 70°C, (k) 1. R_3Cl, 2. Ag_2O, 3. HCl, 4. ^{t}BuOCl, MeOH, 0°C.

Dialkylamine substitution of **6** produced two regioisomers which were identified as **12a-e** and **13a-e** as shown in Scheme 5. The isomers were separated chromatographically and only the oxazolidinones carried forward.

5,5-Spirooxazolidinone

5,5-Spirooxazolidin-2-one **1g** was prepared starting with compound **15**. After cyclization of compound **14**, the amine was quaternized and the oxazolidinone *N*-chlorinated under standard chlorination conditions.



Scheme 3: Synthesis of 5,5-spirooxazolidin-2-one **1g**.

Conditions: (a) EtOAc, LHMDS, -78°C then LiOH (b) DPPA, 110°C then HCl; (c) MeI, Cs_2CO_3; (d) ^{t}BuOCl, 0°C

Imidazolidin-2-one

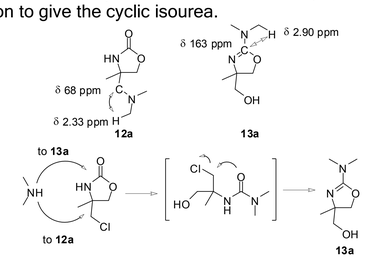
3-Chloroimidazolidin-2-one **2a** was synthesized as shown in Scheme 4. Cyclization of nitro-Henry adduct **17** followed by quaternization of the tertiary amine and chlorination of the imidazolidinone afforded compound **2a**.

Scheme 4: Synthesis of 4,4-disubstituted imidazolidin-2-one **2a**.

Conditions: (a) CH_2O, $H_2NCH_2CH_2NMe_2$; (b) H_2 (500 psi), Raney Nickel; (c) CDI; (d) 1. MeI, 2. Ag_2O, AcOH, 3. HCl; (e) ^{t}BuOCl, 0°C.

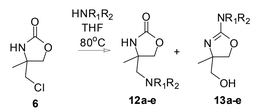
Oxazolidinone Rearrangement

The identities of **12a** and **13a** were established by HMBC spectroscopy. Non-trivial ^1H-^{13}C correlations are shown in Scheme 5. A proposed mechanism of formation for **13a** involves attack of the dimethylamine nucleophile on the oxazolidinone, followed by re-cyclization to give the cyclic isourea.



Scheme 5: Key HMBC correlations in **12a** and **13a** and proposed mechanism of formation of **13a**.

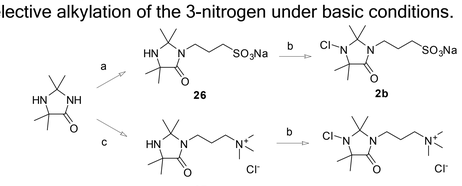
Distribution of products between **12** and **13** varied by amine and the yield of **12** was improved by the addition of NaI.



Entry	R^1, R^2	Additive	Yield (**2**)	Yield (**3**)
a	-Me, -Me	none	30%	41%
a	-Me, -Me	NaI	40%	41%
b	-Et, -Et	none	3%	19%
b	-Et, -Et	NaI	39%	51%
c	$-(CH_2)_4-$	NaI	35%	60%
d	$-(CH_2)_5-$	NaI	49%	36%
e	-H, ^{t}Bu	none	trace	trace

Imidazolidin-4-ones

1-Chloroimidazolidin-4-ones were synthesized from known compound 2,2,5,5-tetramethylimidazolidin-4-one [3] through selective alkylation of the 3-nitrogen under basic conditions.



Scheme 8: Synthesis of 3,3,5,5-tetramethyl imidazolidinones **2b-c.**

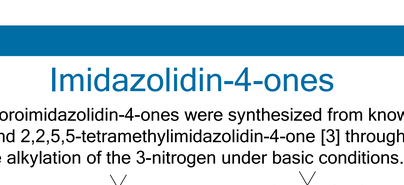
Conditions: (a) $BrCH_2CH_2SO_3Na$, NaH, DMF; (b) ^{t}BuOCl, MeOH, 0°C; (c) $Br(CH_2)_3NMe_3^+Br^-$, NaH, DMF; then Ag_2O, H_2O; then HCl

Hydantoins

1-Chlorohydantoins were synthesized from commercially available 5,5-dimethylhydantoin by alkylation of the imide nitrogen with a water solubilizing functionality, followed by chlorination of the amide nitrogen. Both the sulfonic acid and trimethylammonium solubilizing groups were explored.

Scheme 6: Synthesis of 5,5-dimethyl hydantoins **3a-b**.

To test the effect of different substitutions, a chlorohydantoin with a 5-position water solubilizing group was also synthesized.



Scheme 7: Synthesis of 3,5-dimethyl hydantoin **3c**.

Conditions: (a) 1. $BrCH_2CH_2Cl$, 2. KSAc, 80°C, 3. H_2O_2, HCO_2H; (b) ^{t}BuOCl, 0°C; (c) $BrCH_2CH_2NMe_3^+Br^-$, then Ag_2O, then HCl; (d) $(NH_4)_2CO_3$, NaCN; (e) 6 M HCl; (f) MeI, Cs_2CO_3; (g) HOCl, pH 5, H_2O

Phthalimidoacetone was cyclized with ammonium carbonate and sodium cyanide. Unfortunately, the phthalimide did not survive cyclization conditions and subsequently had to be removed under harsh acidic conditions. The amine and imide were permethylated under basic conditions, and after exchanging the iodide for chloride, the amide was chlorinated with *tert*-butylhypochlorite to give **3c**.

References

1. (a) Wang, L.; Khosrovi, B.; Najafi, R. *Tetrahedron Lett.* **2008**, 49, 2193-2195. (b) Low, E.; Nair, S.; Shiau, T.; Belisle, B.; Debabov, D.; Celeri, C.; Zuck, M.; Najafi, R.; Georgopapadakou, N.; Jain, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 196-198. (c) Francavilla, C.; Low, E.; Nair, S.; Kim, B.; Shiau, T.P.; Debabov, D.; Celeri, C.; Alvarez, N.; Houchin, A.; Xu, P.; Najafi, R.; Jain, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2731-2734.
2. Calvo, P.; Crugeiras, J.; Rios, A.; Rios, M.A. *J. Org. Chem.* **2007**, *72*, 3171-3178.

Biological Activity

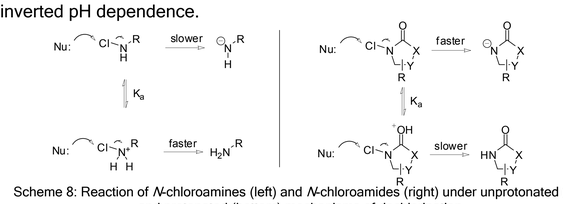
Minimum Bactericidal Concentrations (MBCs) were determined using a modified CLSI M26-A protocol where Mueller-Hinton broth was replaced with isotonic saline (pH 4) or phosphate-buffered saline (pH 7) and the residence time of the compound was reduced to 1 h. Organisms are *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *C. albicans* ATCC 10213.

	MBC, pH 4 (ug/ml)			MBC, pH 7 (ug/ml)		
Entry	*E. coli*	*S. aureus*	*C. albicans*	*E. coli*	*S. aureus*	*C. albicans*
1a	256	256	>512	128	256	1,024
1b	64	256		16	32	128
1c	16	32	512	2	4	16
1d	16	64		2	4	8
1e	16	64		2	2	8
1f	32	128		4	4	16
1g	256	512		16	32	256
2a	512	128				>2,048
2b	4	16		32	64	256
2c	4	2		256	256	
3a	128	128	128	64	64	256
3b	128	>128	>1,024	4	16	128
3c	16	64	>256			
4a	2	2	32	256	512	2048
4b				1,024	>4,096	>4,096

Results and Discussion

Compared to *N*-chloroamines **4a** and **4b**, most of the tested compounds perform worse at pH 4. However, anionic oxazolidinones (**1a-b**) and anionic hydantoin **3a** show pH-independent activity, while their cationic counterparts (**1c-g**, **3b**) show pH-dependent activity in the opposite direction of the chloroamines.

Reaction of chloroamines proceeds via protonation of the chloroamine (pK_a = 6) followed by nucleophilic attack on the protonated species [2]. However, nucleophilic attack on the unprotonated heterocycles is possible due to the reduced pK_b of the amide anion. As cationic water-solubilizing groups would further stabilize the amide anion and as a result show inverted pH dependence.



Scheme 8: Reaction of *N*-chloroamines (left) and *N*-chloroamides (right) under unprotonated (top) and protonated (bottom) mechanisms of dechlorination.

Not surprisingly, cyclic amines **2b-c** have the same pH dependence as their acyclic counterparts, showing enhanced activity at pH 4 relative to pH 7.

Conclusions

We have described a class of water-soluble *N*-chlorinated heterocycles which are active against bacteria (*S. aureus*, *E. coli*) and yeast (*C. albicans*) and whose activities either do not change with pH or increase with increasing pH. These physiochemical properties make them ideal candidates for topical applications in which physiological pH must be maintained.

Comparison of anionic and cationic water solubilizers suggest that the mechanism of reaction follows an unprotonated pathway, in contrast to the chloroamines in which literature has shown the mechanism of reaction to involve protonation of the chloroamine.