Bioorganic & Medicinal Chemistry Letters 21 (2011) 3682–3685

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure stability/activity relationships of sulfone stabilized *N,N*-dichloroamines

Eddy Low, Bum Kim, Charles Francavilla, Timothy P. Shiau, Eric D. Turtle, Donogh J. R. O'Mahony, Nichole Alvarez, Ashley Houchin, Ping Xu, Meghan Zuck, Chris Celeri, Mark B. Anderson, Ramin (Ron) Najafi, Rakesh K. Jain *

NovaBay Pharmaceuticals Inc., 5980 Horton St., Suite 550, Emeryville, CA 94608, United States

ARTICLE INFO

Article history:
Received 14 March 2011
Revised 13 April 2011
Accepted 19 April 2011
Available online 24 April 2011

Keywords:
Antimicrobial
N-Chloroamine

ABSTRACT

Structure stability/activity relationships (SXR) of a new class of *N,N*-dichloroamine compounds were explored to improve antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* while maintaining aqueous solution stability. This study identified a new class of solution-stable and topical antimicrobial agents. These agents are sulfone-stabilized and possess either a quaternary ammonium or sulfonate appendages as a water solubilizing group. Several unique challenges were confronted in the synthesis of these novel compounds which are highlighted in the discussion.

Many antimicrobial compounds used for the prevention or treatment of infections have been rendered less effective through evolved bacterial drug resistance. This has engendered both an urgent need and widespread interest in new, fast-acting, broad spectrum, topical antimicrobials with reduced potential for inducing resistance.[1]

The chlorinated derivatives of taurine, *N*-chlorotaurine (**1**) and *N,N*-dichlorotaurine (**2**) (Fig. 1) are part of the innate mammalian response to infection, produced as antimicrobials to destroy invading microorganisms and protect the body.[2] Myeloperoxidase (MPO; EC 1.11.1.7) in human granulocytes and monocytes uses hydrogen peroxide and chloride to generate hypochlorous acid, which reacts with taurine to produce the longer lived antimicrobial *N*-chlorotaurine.

N-Chlorotaurine has evolved as a natural antimicrobial compound with no known bacterial resistance,[3a] and provides a unique starting point for novel antibiotic drug development. Nagl et al., have previously reported the anti-microbial activity of *N*-chlorotaurine.[3b–g] However, its commercial utility may be limited due to its short shelf-life in solution.[4] Fortunately, we have determined that key properties of chloramine-based antimicrobials can be tailored through the molecule's core targeting moiety (CTM), allowing us to regulate the biological activity, toxicity, and physiochemical properties such as reactivity, stability, and solubility. These *N,N*-dichloramines are a promising class of antimicrobials with a unique mechanism of action (MOA). We are in the process of elucidating the MOA and our studies so far indicate a very rapid inactivation of sulfur containing proteins resulting in their dysfunction, dysregulation or shedding from the membrane leading to the death of the pathogen. A dichloroamine compound identified from this program, NVC-422[5] (**3**) is currently in phase 2 clinical trials for impetigo and viral conjunctivitis.

In previous SXR (structure stability/activity relationship) studies of *N,N*-dichloroamine compounds,[6a,c] we replaced the sulfonate portion of **3** with alternative water solubilizing groups, such as trialkylammonium (**4**),[6c] dimethylsulfonium, pyridinium, and other positively charged heterocycles. Only a limited number of

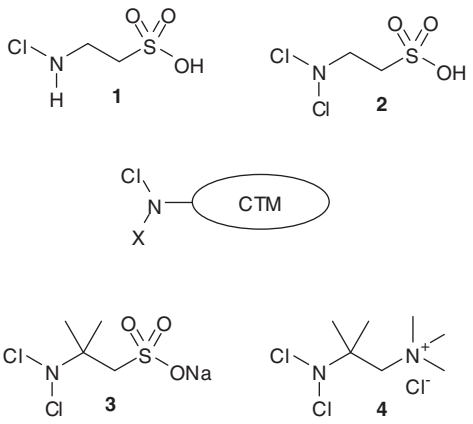


Figure 1. Examples of stabilizing core targeting moieties.

* Corresponding author.
E-mail address: rjain@novabaypharma.com (R.K. Jain).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.084

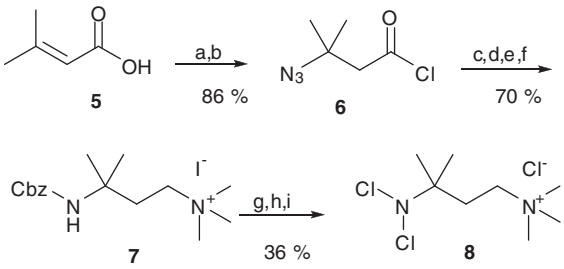
derivatives could be prepared with acceptable levels of solution stability, leaving little room for SAR expansion in this series. We observed that an electron withdrawing (EW) group proximal to the nitrogen is required to stabilize the N–Cl bond, and developed methods to prepare new derivatives with EW functional groups, such as esters, amides, ethers or sulfones, within the scaffold.[6a,c] In particular, a sulfone analog showed good aqueous stability prompting us to explore this functionality further. In the present study, we modified the structural elements of the CTM by varying the linkages between stabilizing, solubilizing and reactive functionalities to monitor their effects on stability and the in vitro anti-microbial activity towards Gram-negative and Gram-positive bacteria as well as fungi.

The relationship of compound stability and activity *vs* the placement of electron withdrawing functionalities within the CTM was examined in both sulfone and quaternary ammonium series. Initially a series of homologous quaternary ammonium compounds was prepared. The synthesis of **8**, a one carbon homolog of **4**, is presented in Scheme 1.

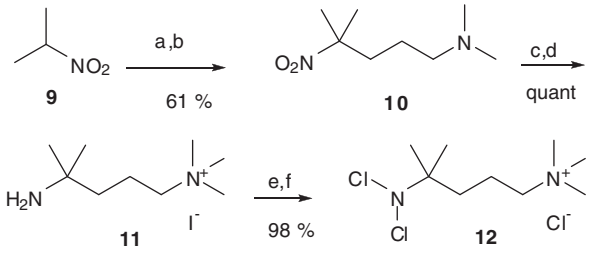
Intermediate **7** was synthesized through the amidation of acid chloride (**6**) with dimethylamine, reduction of the azide and amide, *N*-protection of the primary amine as the benzyloxycarbonyl, and quaternization with methyl iodide. Halogen exchange with silver oxide converted an iodide salt, **7**, to a chloride salt which was more amenable for hydrogenation. The resulting free amine was chlorinated with *t*-BuOCl to obtain dichloroamine product **8**.

The synthesis of the two carbon homolog **12** is shown in Scheme 2. Intermediate **10** was synthesized by a modified version of the reported procedure[7] wherein a conjugate addition of 2-nitropropane to *N*,*N*-dimethylacrylamide was employed, followed by reduction of the amide with borane, to provide the amine. N-Methylation and reduction of the nitro group provided the key intermediate **11**. The nitro group required high pressure hydrogenation for both the chloride or iodide counter ions. Although removal of the iodide was not necessary for reduction to occur, it was necessary to prevent the formation of mixed dihaloamines during the N-chlorination reaction with *t*-BuOCl to afford **12**.

The synthesis of sulfone analogs is presented in Scheme 3. A common intermediate **14** allowed the preparation of compounds having either a sulfonic acid or a trimethylammonium moiety as a terminal water solubilizing group. Compound **14** was prepared from the previously synthesized[6a] azido carboxylic acid **13** in five steps. Alkylation of thiol **14** with the appropriate bromochloroalkane, a common step for analogs **15**, **17**, and **19**, followed by reaction with KSAc and oxidation with formic peracid (from hydrogen peroxide in formic acid) gave the desired sulfone-containing sulfonic acid derivatives, with the exception of **19** which required a repeat of steps f and d prior to oxidation. The syntheses of **21** and **23** were accomplished by treating **14** with the appropriate bromoalkyltrimethylammonium salt. Oxidation of



Scheme 1. Reagents and conditions: (a) NaN_3, AcOH, H_2O, 95 °C, 48 h; (b) $SOCl_2$, DCE, reflux, 4 h; (c) dimethylamine, DCM, ice-cooled, 2 h; (d) LAH, THF, 70 °C, 8 h; (e) THF, Cbz-OSu, 20 °C, 16 h; (f) MeI, EtOH, 20 °C, 16 h; (g) Ag_2O, water, 0.5 h; then aq HCl; (h) 10% Pd/C, MeOH, H_2, 20 °C, 16 h; (i) MeOH, *t*-BuOCl, 0–20 °C, 1 h.



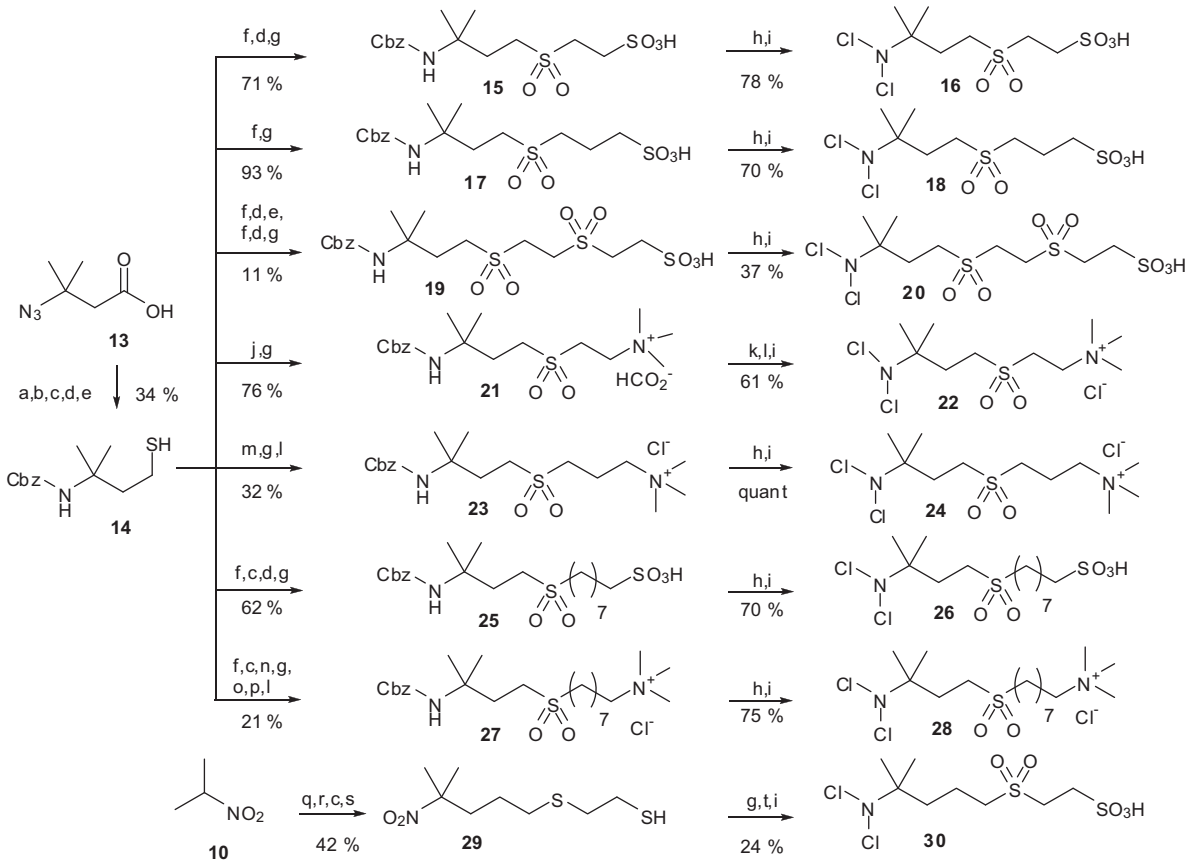
Scheme 2. Reagents and conditions: (a) *N*,*N*-dimethylacrylamide, $BnN^+Me_3OH^-$, dioxane, 100 °C, 1 h; (b) BH_3·THF, 70 °C, 2 h; (c) MeI, MeOH, 20 °C, 48 h; (d) Raney Ni, H_2 (500 psi), MeOH-H_2O, 20 °C, 72 h; (e) Ag_2O, aq HCl, 1 h; (f) MeOH, *t*-BuOCl, 0–10 °C.

the intermediate thioethers gave the desired sulfones **21** and **23**, respectively. The bromide counter ion was converted to bromine during oxidation and was distilled off while concentrating the reaction mixture. Removal of the Cbz group from **21** was accomplished with HBr in acetic acid followed by halogen exchange in the presence of acetic acid. Acetic acid was beneficial in that it both assisted in the dissolution of the Ag_2O and kept the solution acidic.

Under basic conditions **21** is prone to beta elimination to produce a vinyl sulfone side product. While hydrogenation of **21** over Pd/C also caused the elimination of trimethylamine, compound **23** proved to be stable to these conditions and the resultant amine was chlorinated to give dichloroamine product **24**. Compound **25** was synthesized through alkylation of **14** with 8-bromo-octan-1-ol, mesylation of the resulting alcohol, thioacetate displacement of the mesylate and oxidation of the thioether to produce the sulfone-containing sulfonic acid **25**, which was deprotected and chlorinated to give **26**. Compound **27** was synthesized from **14** via the thioether mesylate intermediate, analogous to **25**, where the mesylate was then displaced with potassium phthalimide. The thioether was oxidized to the sulfone followed by *N*-phthalimide deprotection of the amine with hydrazine. Methylation of the amine to the trimethylammonium salt, then halogen exchange and N-chlorination gave **28**. The two-carbon homolog of the sulfone sulfonic acid **30** was obtained from the key intermediate **29**.

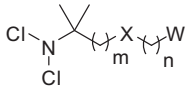
Michael addition of 2-nitropropane to methyl acrylate followed by reduction of the nitro ester to a nitro alcohol, which was mesylated and reacted with sodium 2-mercaptoethanethiolate resulted in an inseparable mixture of disulfides. Oxidation of the crude mixture with formic peracid gave the desired sulfone-containing sulfonic acid in low yield. Hydrogenation of the nitro group under high pressure followed by N-chlorination gave **30**. The data in Table 1 summarizes the antimicrobial activity as MBC or MFC values against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, as well as aqueous solution stability at 40 °C for pH 4 (acetate) and pH 7 (phosphate) buffered saline solutions. All analogs showed comparable antibacterial activity, 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 8 μg/mL in the case of **26** to 1024 μg/mL for compound **18**.

The quaternary ammonium series (QA) (**4**, **8**, and **12**) exhibited excellent solution stabilities where **12** ≪ **8** < **4**. The general trend where solution stability increased as an EW group was moved closer to the N–Cl bond was also observed in a related sulfonic acid series, where **3** was more stable than the corresponding homologs for $m = 2$ and 3.[6a] This observation may be explained through the inductive effect of the EW group, which was consistent with both cationic and anionic EWGs. The reactivity and stability of the N–Cl bond may be related to the electronegativity of the nitrogen. One might expect that the inductive effect of nearby EW group would weaken the N–Cl bond and decrease stability, but this was



Scheme 3. Reagents and conditions: (a) LAH, ether, 0–25 °C, 16 h; (b) Cbz-OSu, isopropanol–H_2O, 16 h; (c) $MeSO_2Cl$, CH_2Cl_2, Et_3N, 0 °C, 2 h; (d) KSAc, DMF, 20–70 °C, 16 h; (e) MeOH, aq NaOH, 1 h; (f) 1-bromo-2-chloroethane for **15** and **19**, sodium 3-bromopropane-1-sulfonate for **17**, 1-bromo-octan-8-ol for **25** and **27**, Cs_2CO_3, DMF–water, 20 °C, 16 h; (g) HCO_2H, 30% H_2O_2, 20 °C, 2 h; (h) 10% Pd/C, MeOH, H_2, 20 °C, 16 h; (i) *t*-BuOCl, 0–10 °C, 1 h; (j) 2-bromo-*N*,*N*,*N*-trimethylethan-1-ammonium bromide, Cs_2CO_3, DMF–water, 20 °C, 16 h; (k) 33% HBr AcOH; (l) Ag_2O, AcOH, water, 0.5 h; then aq HCl; (m) 3-bromo-*N*,*N*,*N*-trimethylpropan-1-ammonium bromide, Cs_2CO_3, DMF–water, 20 °C, 16 h; (n) KPhthalimide, DMF, 50 °C; (o) NH_2NH_2, EtOH, reflux 6 h; (p) MeI, EtOH, 20 °C; (q) methyl acrylate, $BnN^+Me_3OH^-$, dioxane, 100 °C, 1 h; (r) $LiBH_4$, MeOH, 20 °C, 16 h; (s) sodium 2-mercaptoethanethiolate, EtOH, 90 °C, 0.5 h; (t) Raney Ni, H_2 (500 psi), MeOH–H_2O, 20 °C, 72 h.

Table 1
Biological activity of compound **3** and its analogs

Compds					MBC or MFC (μg/mL)[a]			Solution stability (days)[c]	
	m	X	*n*	W	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*C. albicans* ATCC 10231	$t_{1/2}$ pH 4 (saline)	$t_{1/2}$ pH 7 (phosphate)
3	1	n/a	0	SO_3H	2	2	32	>730	>300
4[4c]	1	n/a	0	NMe_3^+	16	8	128	>325	>396
8	2	n/a	0	NM_3^+	2	2	64	>281	>210
12	3	n/a	0	NM_3^+	8	2	256	~80	56
16	2	SO_2	2	SO_3H	8	2	16	>342	>342
18	2	SO_2	3	SO_3H	2	2	1024	>112	85
20	2	$SO_2CH_2CH_2SO_2$	2	SO_3H	2	4	32	>73	14[b]
22	2	SO_2	2	NM_3^+	8	2	32	6	0
24	2	SO_2	3	NM_3^+	8	2	32	>160	70[b]
26	2	SO_2	8	SO_3H	2	4	8	182	70[b]
28	2	SO_2	8	NM_3^+	8	8	32	127	74
30	3	SO_2	2	SO_3H	8	2	128	45	45[b]
31[3a]	1	SO_2	2	SO_3H	4	8	64	>93	1

[a] Minimum Bactericidal Concentration (MBC) was determined using a modified standard method described in CLSI M26-A whereby isotonic saline at pH 4 is substituted for Mueller-Hinton broth (MHB) 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] In 0.6% borate buffer pH 7.

[c] Represents last data point taken from for each compound.

not observed experimentally. Similarly, as the kinetic reactivity of chloramines with nucleophiles is affected by proton transfer in the transition state, it is possible that the EW groups reduce the basicity of the Cl–N group, limiting protonation and thereby increasing

kinetic stability; but again, this is not supported by experimental evidence. Under the more acidic conditions (pH 4 vs 7), the compounds are more stable in solution although they are still more reactive towards other nucleophiles at the lower pH. Other factors play a significant role in stabilizing the dichloroamine function. We observe an optimal distance between the electron-withdrawing group and the dichloroamine of about three bonds; lengthening or shortening the chain leads to instability of the dichloroamine. This compound instability suggests there is an associated increase in reactivity or biological activity. Examination of the QA series (**4**, **8**, and **12**) data show similar activity for all organisms with a tight range of 2- to 4-fold difference between these analogs and demonstrates there is no correlation between compound stability and biological activity for this series. Comparison of the stability of the sulfone-containing sulfonic acids of varying $(CH_2)_m$ chain length showed compound **16**, with a two-methylene spacer, was the most stable (**31** < **30** < **16**), in contrast to the previous two series where the one-carbon spacer **3** and **4** were the most stable. When the $(CH_2)_m$ chain length was held constant and the chain length between the sulfonic acid and the sulfone were varied, **16** was again the most stable (**18** ≈ **26** < **16**). Varying the chain length from n = 3–8 did not affect aqueous stability of either the sulfonic acid or the trimethylammonium series (**18** ≈ **26**, **24** ≈ **28**). Comparison of the analogs when m = 2 $(CH_2)_m$ while switching the WSG showed that the sulfonic acids were generally more stable than the ammonium salts (**22** < **16**, **28** < **26**). When the stabilizing group and the WSG (NMe_3) are too close, the ethylene protons are prone to β-elimination and this leads to the rapid decomposition of **22** to give trimethylamine and a vinyl sulfone.[8] Inserting a second sulfone moiety into the backbone (**20**) decreased stability as compared to a simple alkyl chain (**26**).

While the activity of sulfone-containing sulfonic acid analogs against *C. albicans*, when $(CH_2)_n$ was held constant, also follows the earlier trend, where the analog with n = 2 had the best activity (**30** < **31** < **16**). This was not the case when $(CH_2)_m$ was held constant; there was a 32-fold difference in activity going from (**18**) n = 3 to (**26**) n = 8. There is no obvious explanation for this observation. However, for the sulfone trimethylammonium analogs (SA) (**22**, **24**, and **28**) their activity remained constant. This may allude to a preferential association to *C. albicans*, since there is a known negative charge on the surface of the cell wall.[9] The invariance in activity of SAs (**22**, **24**, and **28**) and dependence of QAs (**4**, **8**, and **12**) activity on chain length against *C. albicans* shows that it is possible to uncouple aqueous stability from activity. The factors governing stability are still under investigation.

In summary, we have described the synthesis and SXR of sulfone-containing analogs of taurine-based chloroamines compared to sodium 2-dichloramino-2-methylpropane-1-sulfonate (**3**). The optimal linker length of 2 has been identified which provides solution stability in the sulfone series. This study also highlights the significant challenges of transforming the amine precursors to the various chloramines. Utilizing these active, stabilizing, structural elements for future dichloroamine design allows us to incorporate additional targeting functions or physiochemical modifying substituents for use as new antimicrobial agents in the treatment of resistant and non-resistant pathogens.

Acknowledgments

The authors wish to acknowledge Drs. John Bartell, Masood Chowhan, Howard Ketelson, and David Stroman for helpful discussions. This work was supported by Alcon Laboratories, Inc.

References and notes

1. (a) Brown, M. L.; Aldrich, H. C.; Gauthier, J. J. *Appl. Environ. Microbiol.* **1995**, *61*, 187; (b) Cookson, B. D.; Bolton, M. C.; Platt, J. H. *Antimicrob. Agents Chemother.* **1991**, *35*, 1997; (c) Brooks, S. E.; Walczak, M. A.; Hameed, R.; Coonan, P. *Infect. Control Hosp. Epidemiol.* **2002**, 692; (d) Suller, M. T. E.; Russell, A. D. *J. Antimicrob. Chemother.* **2000**, *46*, 11; Chuanchuen, R.; Karkhoff-Schweizer, R. R.; Schweizer, H. P. *Am. J. Infect. Control* **2003**, *31*, 124; (e) Mori, T.; Shimizu, N.; Onishi, M.; Sanada, S.; Koda, Y.; Inoue, M.; Kurono, S.; Okada, H. *Jpn. J. Hosp. Pharm.* **2000**, *26*, 652; (f) Russell, A. D.; Tattawasart, U.; Maillard, J.-Y.; Furr, J. R. *Antimicrob. Agents Chemother.* **1998**, *42*, 2151; (g) McCay, P. H.; Ocampo-Sosa, A. A.; Fleming, G. T. A. *Microbiology* **2010**, *156*, 30; Mangalappalli-Illathu, A. K.; Korber, D. R. *Antimicrob. Agents Chemother.* **2006**, *50*, 3588.
2. Nagl, M.; Hess, M. W.; Pfaller, K.; Hengster, P.; Gottardi, W. *Antimicrob. Agents Chemother.* **2000**, *44*, 2507.
3. (a) Nagl, M.; Gottardi, W. *Hyg. Med.* **1996**, *21*, 597; (b) Nagl, M.; Larcher, C.; Gottardi, W. *Antiviral Res.* **1998**, *38*, 25; Nagl, M.; Hess, M. W.; Pfaller, K.; Hengster, P.; Gottardi, W. *AAC* **2000**, 2507; (c) Nagl, M.; Larcher, C.; Gottardi, W. *Antiviral Res.* **1997**, *18*, 577; (d) Nagl, M.; Gottardi, W. *Hyg. Med.* **1992**, *17*, 431; (e) Yazdanbakhsh, M.; Eckmann, C. M.; Roos, D. *Am. J. Trop. Med. Hyg.* **1987**, *37*, 110; (f) Nagl, M.; Hengster, P.; Sementiz, E.; Gottardi, W. *J. Antimicrob. Chemother.* **1999**, *43*, 805; (g) Arnitz, R.; Sarg, Bettina.; Helmut, W. O.; Neher, A.; Lindner, H.; Nagl, M. *Proteomics* **2006**, *6*, 865; (h) Nagl, M.; Lass-Florl, C.; Neher, A.; Gunkel, A.; Gottardi, W. *J. Antimicrob. Chemother.* **2001**, *47*, 871.
4. *N*-Chlorotaurine is stable at refrigerator temperature (2–5 °C). (a) Neher, A.; Nagl, M.; Schrott-Fischer, A.; Ichiki, H.; Gottardi, W.; Gunkel, A. R.; Stephan, K. *Arch. Otolaryngol. Head Neck Surg.* **2001**, *127*, 530.
5. Wang, L.; Khosrovi, B.; Najafi, R. *Tetrahedron Lett.* **2008**, *49*, 2193.
6. (a) 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; (b) Shiau, T. P.; Houchin, A.; Nair, S.; Xu, P.; Low, E.; Najafi, R.; Jain, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1110; (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.
7. Moffett, R. B. *Org. Synth. Coll. Vol.* **1963**, *4*, 652. ; **1952**, *32*, 86..
8. Kroll, F. E. K.; Morphy, R.; Rees, D.; Gani, D. *Tetrahedron Lett.* **1997**, *38*, 8573.
9. Hobden, C.; Teevan, C.; Jones, J.; O'Shea, P. *Microbiology* **1995**, *141*, 1875.