Bioorganic & Medicinal Chemistry Letters 23 (2013) 5650–5653

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Sulfonyl-polyol *N,N*-dichloroamines with rapid, broad-spectrum antimicrobial activity

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ARTICLE INFO

Article history:
Received 12 July 2013
Accepted 5 August 2013
Available online 17 August 2013

Keywords:
Bactericidal
Virucidal
Bacteria
Virus
Conjunctivitis
Chloroamines

ABSTRACT

The discovery and development of antimicrobial agents that do not give rise to resistance remains an ongoing challenge. Our efforts in this regard continue to reveal new potential therapeutic agents with differing physicochemical properties while retaining the effective *N,N*-dichloroamine pharmacophore as the key antimicrobial warhead. In this Letter, we disclose agents containing polyol units as a water solubilizing group. These sulfonyl-polyol agents show broad spectrum bactericidal and virucidal activity. These compounds show 1h MBC's of 16–512 μg/mL against *Escherichia coli* and 4–256 μg/mL against *Staphylococcus aureus* at neutral pH, and 1-h IC_{50}'s of 4.5–32 μM against Adenovirus 5 and 0.7–3.0 μM against Herpes simplex virus 1. The lead compounds were tested in a tissue culture irritancy assay and showed only minimal irritation at the highest concentrations tested.

Bacteria and viruses are quickly developing resistance to currently marketed drugs.[1] To address this problem, an antimicrobial agent with rapid bactericidal and virucidal activity and low potential for resistance development is desired. Based on *N*-chlorotaurine, a molecule produced by neutrophils,[2] we have reported bactericidal and virucidal activity of *N,N*-dichlorotaurine derivatives with anionic[3] as well as cationic[4] solubilizing groups. We are interested in further examining the role of the water-solubilizing group and structure–activity relationships of neutral solubilizers. In our continuing efforts, we report the discovery of agents which are both potently bactericidal and virucidal and are excellent candidates for the treatment of topical infections with unknown etiology such as infectious conjunctivitis.

Conjunctivitis, commonly called 'pink eye,' is the inflammation of the conjunctiva, and can arise from any one (or a combination) of the following: infectious causes such as viruses (e.g., adenovirus, herpesvirus, coxsackievirus, enterovirus)[5] or Gram-positive or Gram-negative bacteria (e.g., *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Haemophilus influenzae*),[6] or noninfectious causes such as chemical agents (e.g., sodium hydroxide, citric acid) or allergic inflammation. Both viral and bacterial conjunctivitis share many common symptoms, and there is great difficulty in assessing what the causative agent actually is.[7] Although most cases of infectious conjunctivitis are viral,[8] there are no approved drugs for viral conjunctivitis. Practitioners who prescribe antibacterial agents as first-line treatments in these cases risk creating drug-resistant bacteria for no clinical benefit.

An ideal drug to treat infectious conjunctivitis would have the following characteristics: (a) be active against a broad spectrum of bacteria, (b) be active against viruses, (c) applied topically to the eye, (d) do not cause irritation, and (e) do not induce or give rise to resistance even after multiple treatments.

Sulfone-extended backbone analogs previously reported with anionic and cationic water-solubilizing groups prompted us to explore the possibility of neutral, polyol-solubilized analogs as a means of increasing the *c*log*P* while maintaining water-solubility. The key intermediate **3** was synthesized as previously reported[3a]. Alkylation of the sulfide with a haloalkanol or epoxide furnished alcohols **4a–e**, which upon oxidation with mCPBA, afforded sulfone-alcohols **5a–e** (Scheme 1). The sulfone-alcohols were *N*-deprotected by hydrogenation and chlorinated with *tert*-butylhypochlorite to give compounds **1a–e**. The enantiomers of **1e** were synthesized from enantiomerically pure glycidols and were tested separately (see Tables 1 and 2).

Intermediate **3** was also alkylated with alkenes, which were in turn dihydroxylated to the corresponding diols. Treatment of **3** with butadiene monoepoxide provided a 2:1 mixture of **6f** (1° attack of epoxide) and **6g** (2° attack of epoxide), which were separable by silica gel chromatography. The alkylation of **3** by butene and hexene derivatives to give **6h** and **6i** was straightforward. The

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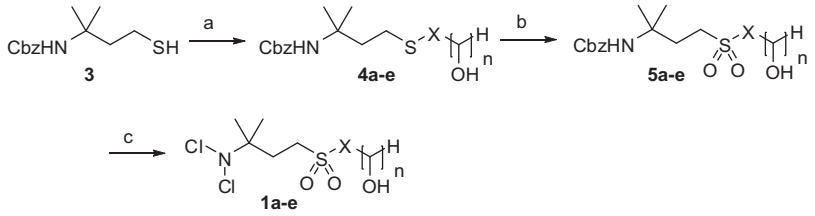
http://dx.doi.org/10.1016/j.bmcl.2013.08.027



Scheme 1. Alkylation of intermediate **3** by alcohol-containing halides and epoxides. Reagents and conditions: (a) alkylating agent, Cs_2CO_3, DMF; (b) *m*CPBA, DCM, 0 °C; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 °C.

Table 1
Compounds synthesized in Scheme 1

Alkylating agent	X	*n*	Entries
I-$(CH_2)_2$OH	CH_2	1	**4a**, **5a**, **1a**
Br-$(CH_2)_3$OH	$(CH_2)_2$	1	**4b**, **5b**, **1b**
Cl-$CH_2C(CH_3)_2CH_2$OH	$CH_2C(CH_3)_2$	1	**4c**, **5c**, **1c**
Br-$(CH_2)_8$OH	$(CH_2)_7$	1	**4d**, **5d**, **1d**
(±)-Glycidol	CH_2	2	**4e**, **5e**, **1e**
(*R*)-Glycidol	CH_2	2	**R-4e**, **R-5e**, **R-1e**
(*S*)-Glycidol	CH_2	2	**S-4e**, **S-5e**, **S-1e**

Table 2
Compounds synthesized in Scheme 2

Alkylating agent	X	Entries
Butadiene monoepoxide	$CH_2CH(OH)$	**6f**, **7f**, **1f**
	$CH(CH_2OH)$	**6g**, **7g**, **1g**
Br-$(CH_2)_2CH{=}CH_2$	$(CH_2)_2$	**6h**, **7h**, **1h**
Br-$(CH_2)_4CH{=}CH_2$	$(CH_2)_4$	**6i**, **7i**, **1i**

oxidation of both the alkene and the sulfide was accomplished with a catalytic OsO_4 procedure employing 3 equiv of NMO. Sulfone-polyols **7f–i** were isolated, **7f** and **7g** as 4:1 and 2:1 mixtures of diastereomers, respectively, which were not separated. Deprotection and *N*-chlorination afforded compounds **1f–i** (Scheme 2).

Intermediate **3** can be alkylated with a wide range of polyhydroxylated compounds. Alkylation with methyl 6-bromo-6-deoxy-α-D-glucopyranoside afforded compound **8**, which was oxidized to **9**, followed by deprotection and *N*-chlorination to give **1j** (Scheme 3).

Two acetylated derivatives of **1e** were synthesized by acetylation of intermediate **5e** (Scheme 4). Treatment with 1 equiv acetic anhydride afforded monoacetyl derivative **10k** which was deprotected and N-chlorinated to give **1k**, while an excess of acetic anhydride afforded diacetyl derivative **10l** which was deprotected and N-chlorinated to give **1l**.

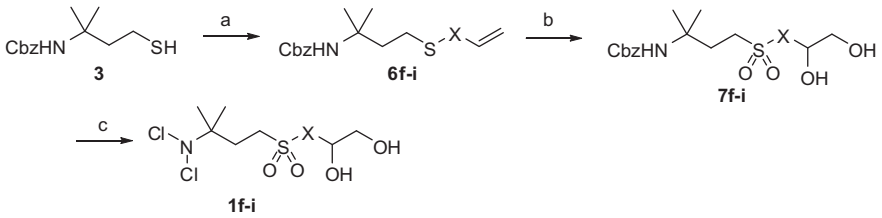
In previous studies,[3b] we have shown that a spacer consisting of two methylenes between a sulfone and dichloroamine was ideal for chemical stability. We also synthesized the one-methylene analog of **1a** to confirm this hypothesis. Previously reported intermediate **11** was alkylated, oxidized, deprotected, and *N*-chlorinated in a sequence similar to that described in Scheme 1 for the synthesis of **1a** (from **3**) to afford **1m** (Scheme 5).

Two sulfonamide analogs **2a** and **2b** were synthesized through the reaction steps as described in Scheme 6. Intermediate **13** was oxidized with aqueous HOCl to afford sulfonyl chloride **14**, which was then reacted with *N*-methylaminoalcohols to give sulfonamides **15a–b**. The sulfonamides were then *N*-deprotected and *N*-chlorinated to give compounds **2a–b**.

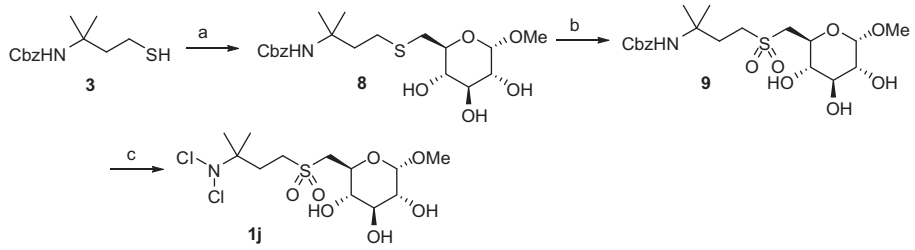
Prior to antimicrobial testing, all compounds were screened for aqueous solution stability. In several cases, the compounds were unstable in aqueous solution and were not tested for *in vitro* antimicrobial activity. In one case (**2b**) the compound was stable in aqueous solution but unstable as a solid.

Susceptibility testing of *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 to **1a–m** and **2a–b** was conducted using modified CLSI methods. CLSI M26-A protocol for minimum bactericidal concentration (MBC) testing was modified by substituting 0.9% acetate saline buffer, pH 4 (ASB) or phosphate-buffered saline, pH 7 (PBS) for Cation-Adjusted Mueller Hinton Broth (CAMHB) to circumvent the reactivity of chloroamine compounds to certain components of CAMHB. To reflect the expected short exposure time in ophthalmic formulations, the MBC assay was shortened from 16–20 h at 35 °C to 1 h at room temperature. The MBC was defined as the lowest concentration achieving >99.9% kill. Microorganisms were first grown to mid-log phase, centrifuged and suspended in ASB or PBS. Next, the organisms were added to serial twofold dilutions of the test compound in ASB or PBS to a final inoculum of 10^5–10^6 CFU/mL and incubated for 1 h at room temperature.Aliquots of the cell suspension were plated on agar with growth media, incubated 24 h at 35 °C and CFUs were quantified.

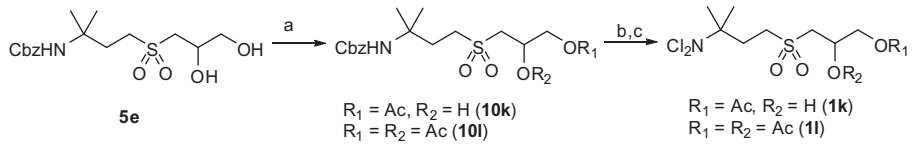
Adenovirus 5 (Ad5, ATCC VR-1516) and Herpes simplex virus 1(HSV-1) (ATCC, strain F, VR-733) stocks were diluted 1:100 in 20 mM PBS or 5 mM ASB supplemented with 2.5% glycerol, aliquoted and stored at −80 °C. Threefold serial dilutions of compounds were prepared in the respective buffer in 96 well plates in quadruplicate. Freshly thawed virus was diluted 1:20 in the same buffer, added 1:1 (v/v) to the diluted compounds and incubated for 60 min at room temperature. Excess compound was neutralized by adding the same volume of 2× neutralization media (2× D-MEM/F12, Invitrogen, Carlsbad, CA) supplemented with 20% FBS, 1.2 g/L $NaHCO_3$, 20 mM L-glutamine and 1000 IU/mL penicillin/100 μg/mL streptomycin (Mediatech)) for 60 min at room temperature. 200 μL of the neutralized virus/compound mixtures



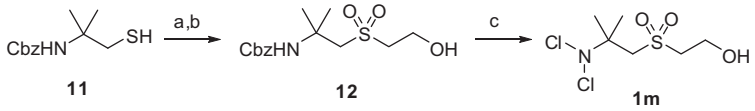
Scheme 2. Alkylation of intermediate **3** with alkene-containing halides and epoxides. Reagents and conditions: (a) alkylating agent, Cs_2CO_3, DMF; (b) OsO_4 (cat.), NMO, acetone; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 °C.



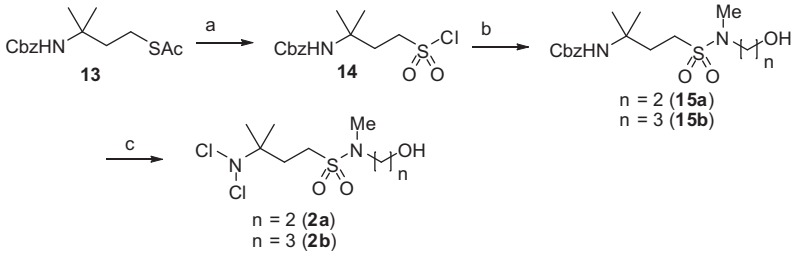
Scheme 3. Alkylation of intermediate **3** by a saccharide derivative. Reagents and conditions: (a) methyl 6-bromo-6-deoxy-α-D-glucopyranoside, Cs_2CO_3, DMF; (b) *m*CPBA, DCM, 0 °C; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 °C.



Scheme 4. Acetylation of **5e** and synthesis of acetylated analogs **1k** and **1l**. Reagents and conditions: (a) 1.2 equiv or 2.5 equiv Ac_2O, pyridine, DMAP, DCM, 0 °C; (b) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 °C.



Scheme 5. Alkylation of intermediate **11** with iodoethanol. Reagents and conditions: (a) ICH_2CH_2OH, Cs_2CO_3, DMF; (b) *m*CPBA, DCM, 0 °C; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 °C.



Scheme 6. Synthesis of sulfonamide derivatives **2a**–**b**. Reagents and conditions: (a) HOCl, H_2O, 0 °C; (b) $MeHN(CH_2)_nOH$; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 °C.

were added to A549 cells for Ad5 or Vero cells for HSV-1 (both ATCC), which were prepared by seeding 5000 cells/well in a flat-bottom 96-well plate on the day before the assay, and incubated for 1–2 h at 37 °C to allow viral adsorption to the cells. Unbound virus was aspirated. Cell culture medium without any compound was added back to the cells. Cells were incubated for 6 days at 37 °C, 5% CO_2, 90% relative humidity. Viral cytopathic effect (CPE) was determined using Dojindo cell counting kit-8 (Rockville, MD) and SpectraMax plate reader (Molecular Devices, Sunnyvale, CA). Cytotoxicity controls without the addition of Ad5 were carried out in parallel. The % inhibition of viral CPE was calculated as follows: (compound-treated infected sample—untreated infected control)/(untreated, noninfected control-untreated, infected control) × 100. The 50% inhibitory concentration (IC_{50}) was calculated by plotting (% inhibition) versus (log compound concentration) in GraphPad Prism 4 (La Jolla, CA) using the sigmoidal dose–response equation with a top value constraint set to 100.

The potential for ocular irritancy of Triton X-100 and compound **1e** were compared. The EpiOcular™ tissue model has a cornea-like three-dimensional architecture and provides a nonanimal alternative to the traditional Draize rabbit eye test with excellent in vitro–in vivo correlation.[9] EpiOcular tissues (Mattek Corp.) were placed in 900 μL cell culture media and 100 μL test compound was added to the apical side of the tissue for varying exposure times. Tissues were rinsed with 1× PBS and placed in an MTT solution for 3 h. Tissues were extracted overnight and tissue viability was determined by MTT absorbance. Tissue viability was correlated with a Draize-type score for tissue irritancy according to Mattek's instructions.

Table 3 summarizes the antimicrobial activity as MBC values against *S. aureus* and *E. coli*, and virucidal activity as IC_{50} values against Ad5 and HSV-1. As expected, all tested compounds showed excellent bactericidal activity at pH 4, most compounds with only two to fourfold variations in activity. The only compound which showed significantly different activity, **1j**, has a molecular weight much higher than the other compounds and the increased value is simply a reflection of its higher molecular weight. All tested compounds also showed excellent virucidal activity at pH 4, most compounds with only twofold variations in activity.

Table 3
Bactericidal and virucidal activities of reported compounds

Entry	pH 4			pH 7			
	MBC *E. coli* (μg/ml)	MBC *S. aureus* (μg/ml)	IC_{50} Ad5 (uM)	MBC *E. coli* (μg/ml)	MBC *S. aureus* (μg/ml)	IC_{50} Ad5 (uM)	IC_{50} HSV-1 (uM)
1a	0.5	1	1.4	8	16	4.5	0.7
1b	*	*	*	*	*	*	*
1c	4	2	1.8	64	128	9.6	0.9
1d	*	*	*	256	128	32	nt
1e	2	2	2.7	16	4	26	1.5
R-1e	2	2	2.2	32	4	14	1.2
S-1e	2	1	2.4	32	4	13	1.0
1f	2	1	nt	128	32	nt	nt
1g	2	2	1.5	64	16	19	3.0
1h	2	1	1.4	64	16	18	1.2
1i	*	*	*	*	*	*	*
1j	8	8	nt	512	256	nt	nt
1k	2	4	nt	64	32	nt	nt
1l	1	2	nt	128	128	nt	nt
1m	1	1	2.3	*	*	*	*
2a	2	1	1.5	64	128	14	1.2
2b	*	*	*	*	*	*	*

nt = not tested. * = not tested due to compound decomposition.

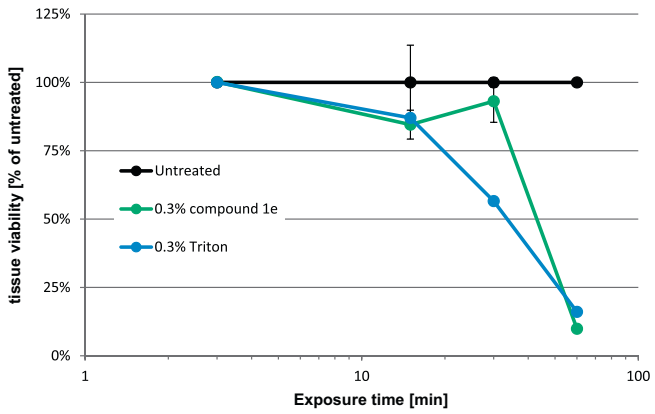


Figure 1. Effects of reported compound **1e** on tissue viability in an EpiOcular model.

In contrast to the strong pH dependence seen for previously reported compounds—256-fold increase in MBC for *E. coli*, 512-fold increase in MBC for *S. aureus*, 22-fold increase in IC_{50} for Ad5—several compounds showed minimal pH dependence between pH 4 and 7. Compound **1a** showed a 16-fold increase in MBCs from pH 4 to pH 7 and a 3.2-fold increase in Ad5 IC_{50}, while compound **1e** showed only a twofold increase in MBC for *S. aureus* and an eightfold increase in MBC for *E. coli*, and a 10-fold increase in Ad5 IC_{50}. Most other compounds showed 32- to 128-fold increase in MBC and 5- to 15-fold increase in IC_{50}.

These results lead us to hypothesize that the ability of compounds **1a** and **1e** to cross lipid bilayers is greatly enhanced due to the lack of charge relative to previously published analogs. As further evidence supporting this hypothesis, we note that all compounds tested against HSV-1, a lipid-enveloped virus, showed 30-fold improvement over other anionic compound (data not shown). Compounds **1a** and **1c** show high nanomolar potency against HSV-1 (Table 3).

In the EpiOcular tissue irritancy model, 0.3% of compound **1e** in 20 mM PBS pH 7 reduced the tissue viability to 50% of the untreated control within 46 min, corresponding to a Draize score of none or minimal irritancy (Fig. 1).

In summary, we have described the synthesis of sulfone-containing analogs with polyols as neutral, water-solubilizing groups for dichloroamines. These molecules have been found to have potent virucidal activity against adenovirus 5 and herpes simplex virus 1 in addition to bactericidal activity against *S. aureus* and *E. coli*. These compounds show relatively little pH dependence compared with anion- and cation-solubilized dichloroamines, having potent activity at pH 7 in addition to pH 4, with compound **1e** showing the least pH dependence of the series. Compound **1e** was shown to be minimially irritating in an EpiOcular tissue model. Expanded preclinical work is being conducted to evaluate the potential for these compounds to enter clinical trials for viral and bacterial conjunctivitis.

Acknowledgments

The authors thank Professor John Soderquist and Dr. Larry Truesdale for their help and support through these studies.

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