MEDI 306

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

Timothy P. Shiau, Eddy Low, Bum Kim, Eric D. Turtle, Charles Francavilla, Donogh J. R. O'Mahony, Lisa Friedman, Louisa D'Lima, Andreas Jekle, Meghan Zuck, Nichole J. Alvarez, Mark Anderson, Ramin (Ron) Najafi and Rakesh K. Jain

NovaBay Pharmaceuticals, 5980 Horton Street, Suite 550, Emeryville, CA 94608

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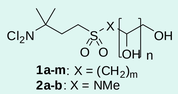
Timothy Shiau
Phone (510)-899 8862
tshiau@novabaypharma.com

Abstract

N,*N*-Dichloroamines with polyol solubilizers have fast-acting, bactericidal and virucidal activity and are suitable for the topical treatment of infectious diseases of the skin. These agents show excellent activity against a broad range of bacterial and viral pathogens, both at pH 4 and pH 7. The sulfonyl-polyol series showed 1-hour MBC's of 16-512 ug/mL against E. coli and 4-512 ug/mL against *S. aureus* at neutral pH, and 1-hour IC_{50}'s of 4.5-80 uM against Ad5 and 0.7-58 uM against HSV-1. The lead compounds were tested in a tissue culture irritancy assay and shows only minimal irritation at the highest concentrations tested.



1a-m: X = $(CH_2)_m$
2a-b: X = NMe

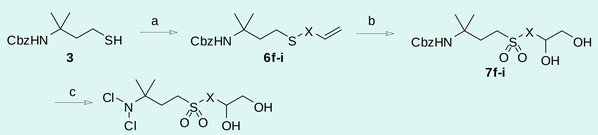
MBC (*E.coli*) 16-512 ug/ml
MBC (*S. aureus*) 4-512 ug/ml
IC_{50} (Ad5) 4.5-80 uM
IC_{50} (HSV-1) 0.7-58 uM

Introduction

Bacteria and viruses are quickly developing resistance to currently marketed drugs. To address this problem, an antimicrobial agent with rapid bactericidal and virucidal activity and low potential for resistance development is desired. *N*,*N*-Dichlorotaurine analogs are based on the naturally occurring *N*-chlorotaurine, which is produced by the body's neutrophils to kill microbial pathogens. The analogs reported within this poster are potential candidates for the treatment of topical infections with mixed viral/bacterial pathogenesis such as epidemic keratoconjunctivitis (EKC).

Synthesis of Polyols by Dihydroxylation

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** (1º attack of epoxide) and **6g** (2º attack of epoxide), which were separable by silica gel chromatography. Alkylation of **3** by butene and hexene derivatives to give **6h** and **6i** was straightforward. Oxidation of both the alkene and the sulfide was accomplished with catalytic OsO_4 and 3 equivalents of NMO; sulfone-polyols **7f-i** were isolated, **7f** and **7g** as 4:1 and 2:1 mixtures of diastereomers which were not separated. Deprotection and *N*-chlorination afforded compounds **1f-i**.



3 **6f-i** **7f-i** **1f-i**

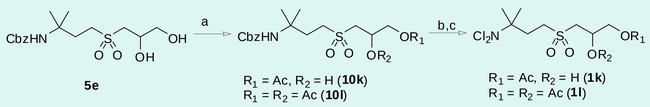
Conditions: (a) alkylating agent, Cs_2CO_3, DMF; (b) OsO_4 (cat.), NMO, acetone; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Alkylating Agent	X	Entry
butadiene monoepoxide	$CH_2CH(OH)$	**1f**
	$CH(CH_2OH)$	**1g**
$Br\text{-}(CH_2)_2CH{=}CH_2$	$(CH_2)_2$	**1h**
$Br\text{-}(CH_2)_4CH{=}CH_2$	$(CH_2)_4$	**1i**

Table 2: Compounds **1f-i**

Acylated Polyols

Two acetylated derivatives of **1e** were synthesized by acetylation of intermediate **5e**. Treatment with 1 eq. 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**.

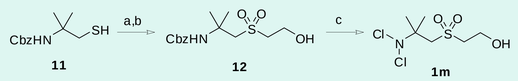


5e
R_1 = Ac, R_2 = H (**10k**)
R_1 = R_2 = Ac (**10l**)
R_1 = Ac, R_2 = H (**1k**)
R_1 = R_2 = Ac (**1l**)

Conditions: (a) 1.2 eq. or 2.5 eq. Ac_2O, pyridine, DMAP, DCM, 0 ºC; (b) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Shortened Linker

In previous studies we have shown that a spacer of two methylenes between a sulfone and dichloroamine was ideal for chemical stability; we 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 for **1a** to afford **1m**.



11 **12** **1m**

Conditions: (a) ICH_2CH_2OH, Cs_2CO_3, DMF; (b) mCPBA, DCM, 0 ºC; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Biological Activity

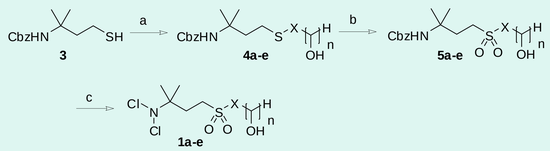
Prior to antimicrobial testing, all compounds were screened for stability. In several cases, compounds were unstable in aqueous solution and were not tested for *in vitro* antimicrobial activity. In one case (**2b**),the compound was stable in aqueous solution but unstable as a solid.

	pH 4			pH 7			
Entry	MBC *E. coli* (ug/ml)	MBC *S. aureus* (ug/ml)	IC_{50} Ad5 (uM)	MBC *E. coli* (ug/ml)	MBC *S. aureus* (ug/ml)	IC_{50} Ad5 (uM)	IC_{50} HSV (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	1	nt	nt
1e	2	2	2.7	16	4	25.5	1.5
R-1e	2	2	2.2	32	4	14.1	1.0
S-1e	2	1	2.4	32	4	13.6	1.2
1f	2	1	nt	128	32	nt	nt
1g	2	2	1.5	64	16	18.6	3
1h	2	1	1.4	64	16	18.1	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	2.2	*	*	*	*
2a	2	1	1.5	64	128	14.0	1.2
2b	*	*	*	*	*	*	*

nt = not tested based on MBC values. * = not tested due to compound stability

Synthesis of Polyols by Alkylation

Intermediate **3** was synthesized as previously reported. Alkylation of the sulfide with a haloalkanol or epoxide furnished alcohols **4a-e**, which on oxidation with mCPBA afforded sulfone-alcohols **5a-e**. The sulfone-alcohols were *N*-deprotected by hydrogenation and chlorinated with *tert*-butylhypochlorite to give compounds **1a-e**. The enantiomers of **1e** were synthesized and tested separately from enantiomerically pure glycidol.



3 **4a-e** **5a-e** **1a-e**

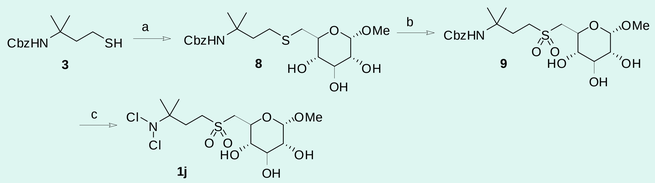
Conditions: (a) alkylating agent, Cs_2CO_3, DMF; (b) mCPBA, DCM, 0 ºC; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Alkylating Agent	X	n	Entry
$I\text{-}(CH_2)_2OH$	CH_2	1	**1a**
$Br\text{-}(CH_2)_{\text{-}3}OH$	$(CH_2)_2$	1	**1b**
$Cl\text{-}CH_2C(CH_3)_2CH_2OH$	$CH_2C(CH_3)_2$	1	**1c**
$Br\text{-}(CH_2)_8OH$	$(CH_2)_7$	1	**1d**
(±)-glycidol	CH_2	2	**1e**
(R)-glycidol	CH_2	2	**R-1e**
(S)-glycidol	CH_2	2	**S-1e**

Table 1: Compounds **1a-e**

Saccharide-linked Dichloroamine

Intermediate **3** can be alkylated with a wide range of alcohols. Alkylation with a protected monosaccharide afforded compound **8**, which was oxidized to **9**, and deprotected/chlorinated to give **1j**.

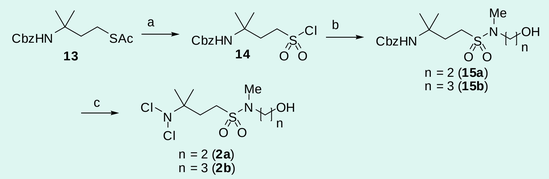


3 **8** **9** **1j**

Conditions: (a) 6-bromo-1-methoxy-a-glucopyranose, Cs_2CO_3, DMF; (b) mCPBA, DCM, 0 ºC; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Sulfonamide Linker

Two sulfonamide-linked analogs were synthesized as well. 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**.

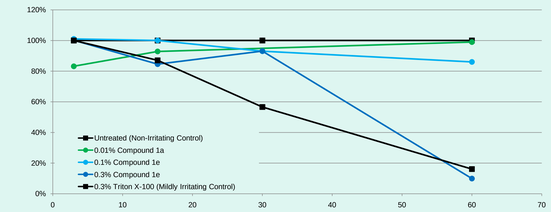


13 **14**
n = 2 (**15a**)
n = 3 (**15b**)
n = 2 (**2a**)
n = 3 (**2b**)

Conditions: (a) HOCl, H_2O; (b) $MeHN(CH_2)_nOH$; (c) H_2 (1.3 atm), 10% Pd/C, MeOH; then *t*-BuOCl, MeOH, 0 ºC.

Irritancy

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 1x PBS and placed in an MTT solution for 3 hours. 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.



Results and Conclusions

- All compounds showed strong bactericidal (*S. aureus* and *E. coli*) and virucidal (adenovirus serotype 5) activity at pH 4, with only minor (2-fold) changes in activity across the series. Compound **1j** displayed decreased activity; however, this may only be a function of the lower molar concentration due to its higher molecular weight.
- Compounds **1a** and **1e** showed minimal pH dependence and were also very active against both bacteria and viruses at pH 7.
- Compounds **1a** and **1c** show high nanomolar potency against HSV-1. All compounds tested in this series against HSV-1, a lipid-enveloped virus, showed extremely potent activity. It may be plausible that electrically-neutral molecules allows permation into lipid bilayers; however, more study is warranted.
- These sulfone-extended, polyol-solubilized dichloroamines have potent virucidal and bactericidal activity. Compounds **1a** and **1e** have been selected for *in vivo* studies against viral conjunctivitis.