Aganocide® Compounds Effective Against Ophthalmic Pathogens

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Abstract

Purpose: There is no effective FDA-approved medication for adenoviral conjunctivitis currently available. Novel treatment options are needed that would target both the viral and bacterial etiological agents of conjunctivitis. In this report, we show results on Aganocide® compounds, a new class of topical microbicides with *in vitro* efficacy against common ophthalmic pathogens such as Adenovirus, Herpes Simplex Virus, *S. aureus, S. marcescens*, *H. influenzae* and *C. albicans*.
Results: We have shown that our first-in-class Aganocide® compound, NVC-422 (2-(dichloroamino)-2-methylpropanesulfonic acid) has broad-spectrum activity against viral, bacterial and fungal ocular pathogens (1). Here, we present new Aganocide® compounds with increased virucidal, bactericidal or fungicidal activity, more favorable cytotoxicity profile or pH-independent activity. NVC-638 (2-(3-(dichloroamino)-3-methylbutylsulfonyl)ethanesulfonic acid) has similar microbicidal activity but a further improved cytotoxicity profile compared to NVC-422. The acyclic hydrophilic compound NVC-727 (3-(3-(dichloroamino)-3-methylbutylsulfonyl)propane-1,2-diol) has better anti-HSV-1 and bactericidal activity over a broad pH range while maintaining the excellent anti-Adenovirus activity of NVC-422. Finally, the cyclic compound NVC-704 (3-(3-chloro-2,2,4,4-tetramethyl-5-oxoimidazolidin-1-yl)propane-1-sulfonic acid) has increased activity against *C. albicans* combined with a favorable cytotoxicity, bactericidal and virucidal profile.
Conclusions: We present novel, fast-acting, broad-spectrum Aganocide® compounds with good solution stability and broad-spectrum activity against viral, bacterial and fungal ophthalmic pathogens. The broad-spectrum *in vitro* activity and good cytotoxicity profile validate Aganocide® compounds as new therapeutic agents for the treatment of both viral and bacterial conjunctivitis.

Materials & Methods

Modified Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) assays. Bacterial and fungal strains were purchased from the ATCC. CLSI protocols for MBC and MFC testing were modified to compensate for the reactivity of Aganocide compounds to certain components of growth media. Due to the rapid cidal nature of Aganocide® compounds, the assays were shortened from 16-20 hours at 35 °C to 60 minutes at room temperature. Microorganisms were grown to mid-log phase, resuspended in 0.9% saline, pH 4 or 20 mM Phosphate-buffered Saline pH7 and added to dilutions of test compounds in the same buffers to a final inoculum of 10^5 – 10^6 CFU/mL. After one hour, aliquots of the reaction mixture were transferred into 9 volumes of D/E neutralizing broth and plated for quantitation. The MBC or MFC is the lowest concentration of test compound that kills > 99.9% of the challenge organism.

Time Kill assay. *Staphylococcus aureus* (ATCC 29213) was grown to mid-log phase, resuspended in 5 mM acetate saline pH 4 in the presence or absence of synthetic or human donor tears (Bioreclamation, LLC) and added to test compounds in to a final inoculum of 10^5 – 10^6 CFU/mL. Aliquots were removed at the indicated time points, neutralized as described above, and plated for quantitation. Results of triplicate independent assays were analyzed and graphed using Prizm® plot.

Virucidal assay. Serially diluted compounds in the indicated buffer were incubated with Ad5 or HSV-1 (ATCC) for 1hr at room temperature. Excess compound was neutralized by adding an equal volume of 2x media (2x D-MEM/F12 supplemented with 20% FBS) for 1hr at room temperature. Virus/compound mixtures were added to A549 cells for Ad5 and Vero cells for HSV-1 and incubated for 1-2 hours at 37°C. Unbound virus was aspirated off. Cell culture medium was added back to the cells. Cells were then incubated for 6 days at 37°C. Viral cytopathic effect was determined using Dojindo cell counting kit-8. The 50 % inhibitory concentration (IC_{50}) was calculated in GraphPad Prism 4 using the sigmoidal dose-response equation.

Cytotoxicity Assay: Compounds were serially diluted in 20 mM Phosphate-buffered Saline, pH7 and added to mouse fibroblast L929 cells for 1h at 37°C. Compound was replaced with cell culture media and cells were incubated overnight at 37°C. Cell viability was determined using Dojindo cell counting kit-8.

EpiOcular™ Tissue Irritancy Assay: EpiOcular tissues (Mattek Corp.) were placed in 900 µl cell culture media and 100 µl 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

Aganocide® Compounds

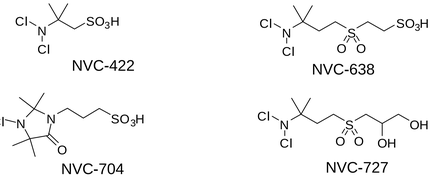


Table 1: Virucidal Activity against Adenovirus 5 and HSV-1

	IC_{50} [µM]#		
Compound	Ad5 at pH4*	Ad5 at pH7**	HSV-1 at pH7**
NVC-422	1.1 ± 0.6	22.9 ± 11.6	34.5 ± 9.3
NVC-638	4.3 ± 3.3	80.8 ± 44.4	58.1 ± 21.9
NVC-704	1.5 ± 0.9	433 ± 235	52.9 ± 4.0
NVC-727	2.7 ± 2.3	29.0 ± 17.6	1.5 ± 0.4

50% inhibitory concentration of n ≥ 2; * Determined in 5 mM Acetate buffered Saline pH4, ** Determined in 20 mM phosphate-buffered Saline, pH7

Table 2: Antimicrobial Activity against *S. aureus, E. coli* and *C. albicans*

	MBC/ MFC [µM]#					
	S. aureus		*E. coli*		*C. albicans*	
Compound	pH4*	pH7**	pH4*	pH7**	pH4*	pH7**
NVC-422	8.2	2098	8.2	1049	131	8793
NVC-638	6.1	n.d.	24.4	n.d.	48.8	n.d.
NVC-704	49.9	199.5	12.5	99.8	> 798$	> 3192$
NVC-727	6.8	6.8	8.0	127.6	> 3480	> 3480

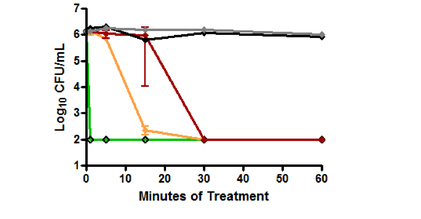
Minimal Bactericidal/Fungicidal Concentration of n ≥ 2;
* Determined in 5 mM Acetate buffered Saline pH4;
** Determined in 20 mM phosphate-buffered Saline, pH7;
$ initial results of MFC of 50 and 798 µM for NVC-704 (as indicated in the abstract) could not be confirmed with later batches.

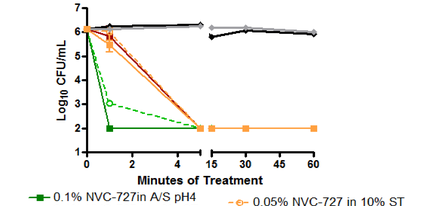
Table 3: Cytotoxicity in mammalian cells and irritancy in EpiOcular™ Tissue

Compound	Draize-type score at 0.3% compound concentration*	CC_{50} [mM]#
NVC-422	non-irritating	6.0
NVC-638	non-irritating	10.2
NVC-704	n.d.	4.6
NVC-727	mildly irritating	0.17

50% cytotoxic concentration measured on mouse fibroblast cell line L929 after 1h exposure in 20 mM Phosphate-buffered Saline, pH7; * Irritancy scores were determined after exposure of EpiOcular™ tissue to 0.3% of the indicated compound for various times and MTT-based cell-viability determination

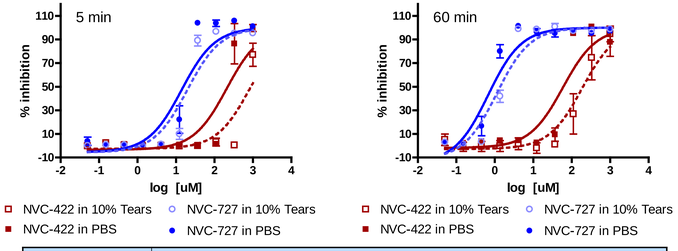
Figure 1: Time-Kill kinetics of 0.3% NVC-422 and 0.1 and 0.05% NVC-727 against *S. aureus 29213* in the presence of 10% synthetic tears (ST)* or human donor tears (HDT).





*synthetic tears: 0.05% lysozyme, 0.05% IgG, 0.05% human albumin, 0.03% $CaCl_2$, 0.036% Na-citrate, 0.14% citric acid, 0.9% NaCl, pH7.4

Figure 2: Inhibition of HSV-1 by NVC-422 and NVC-727 in the presence of 10% synthetic tears



	IC_{50} [µM]#			
	5 min		60 min	
Compound	PBS*	10% synthetic tears**	PBS*	10% synthetic tears**
NVC-422	208	846	58.7	187
NVC-727	14.0	19.7	0.67	1.13

50% inhibitory concentration;
* 20 mM phosphate-buffered saline, pH7;
**synthetic tears: 0.05% lysozyme, 0.05% IgG, 0.05% human albumin, 0.03% $CaCl_2$, 0.036% Na-citrate, 0.14% citric acid, 0.9% NaCl, pH7.4

Conclusions

- The Aganocide® compounds NVC-422 and NVC-727 are, broad-spectrum, fast-acting antimicrobial agents with a good safety profile with a new mechanism of action.
- NVC-727 is a fast-acting antimicrobial agent
  - in the presence of tears,
  - active over a wide pH range, and
  - exhibits good anti-HSV-1 activity.
- These compounds have the potential to be new agents for the treatment of viral and bacterial conjunctivitis.

References

(1) Wang L. et al., 2011: Chemical Characterization and Biological Properties of NVC-422, a Novel, Stable N-Chlorotaurine Analog. Antimicrobial Agents and Chemotherapy. Epub ahead of print.

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