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Disulfiram-based disulfides as narrow-spectrum antibacterial agents

Jordan G. Sheppard [a], Keely R. Frazier [a], Pushkar Saralkar [b], Mohammad F. Hossain [a], Werner J. Geldenhuys [b], Timothy E. Long [a,c,*]

[a] Department of Pharmaceutical Science and Research, School of Pharmacy, Marshall University, Huntington, WV, USA
[b] Department of Pharmaceutical Science, School of Pharmacy, West Virginia University, Morgantown, WV, USA
[c] Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, USA

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ABSTRACT

Sixteen disulfides derived from disulfiram (Antabuse™) were evaluated as antibacterial agents. Derivatives with hydrocarbon chains of seven and eight carbons in length exhibited antibacterial activity against Gram-positive *Staphylococcus, Streptococcus, Enterococcus, Bacillus,* and *Listeria* spp. A comparison of the cytotoxicity and microsomal stability with disulfiram further revealed that the eight carbon chain analog was of lower toxicity to human hepatocytes and has a longer metabolic half-life. In the final analysis, this investigation concluded that the *S*-octylthio derivative is a more effective growth inhibitor of Gram-positive bacteria than disulfiram and exhibits more favorable cytotoxic and metabolic parameters over disulfiram.

Disulfiram (Antabuse™) is an oral prescription drug for the treatment of alcohol abuse disorder.[1] Upon absorption, disulfiram (DSF)[2] and/or its metabolites[3] inhibit aldehyde dehydrogenase (ALDH) enzymes that oxidize acetaldehyde from ethanol metabolism into acetic acid. The inactivation of hepatic ALDH leads to buildup of toxic acetaldehyde in the body, which manifests 'hangover' symptoms (e.g., headache, nausea) to deter alcohol consumption.[4]

By chemical nature, electrophilic (δ+) DSF is readily cleaved by thiol-bearing substances such as cysteine enzymes. The thiol-disulfide exchange reactions result in the simultaneous addition and release of diethyldithiocarbamate (DDTC). In the case of ALDH, *in vitro* studies have shown that a second cysteine residue near the addition site may cleave the labile DDTC adduct with concomitant intramolecular disulfide bond formation (Fig. 1).[2] As a versatile inhibitor of cysteine enzymes, DSF has been investigated as a treatment for other clinical conditions. Recent U.S. clinical trials have evaluated DSF as a repurposed treatment for methamphetamine dependence (NCT00731133), cocaine addiction (NCT00395850), melanoma (NCT00256230), pancreatic cancer (NCT02671890), and HIV infection (NCT01286259).[5] In the area of infectious disease, we recently reported that DSF inhibits the *in vitro* growth of methicillin-resistant *Staphylococcus aureus* (MRSA) at a minimum inhibitory concentration (MIC) range of 4–32 μg/mL and exhibits synergism with vancomycin (VAN) against VAN-resistant *S. aureus* (VRSA).[6] The mechanism of MRSA inhibition was also attributed to the transfer of DDTC from DSF to thiophilic substances involved in the regulation of bacterial cell growth. Due to its labile chemical nature, we hypothesized that replacement of the DDTC component in DSF (Fig. 1) with *S*-alkylthio groups would increase antibacterial activity and metabolic stability.

To test this hypothesis, we first synthesized sixteen DSF-derived asymmetric disulfides (**1a**–**p**) to deduce the relationship of structure on antibacterial activity (Scheme 1). The compounds were readily prepared by a thiol-disulfide exchange reaction between DSF and respective thiol in DMF.[7] Purification by silica gel chromatography afforded the products as nonaromatic oils in a yield range of 32–67% and median yield of 53%. Spectroscopic data and physical characteristics of the compounds were in agreement with previous findings.[8]

Antibacterial testing was performed by the broth microdilution assay in 96-well plate format.[9,10] The test agents were initially evaluated against *Staphylococcus*, *Streptococcus*, and *Enterococcus* spp. as our previous research on DSF indicated that Gram-positive cocci would be susceptible.[6] Table 1 shows the MICs of analogs **1a**–**p** in comparison with DSF and VAN. MRSA and *Staphylococcus epidermidis*

* Corresponding author at: Department of Pharmaceutical Science and Research, School of Pharmacy, Marshall University, Huntington, WV, USA.
E-mail address: longt@marshall.edu (T.E. Long).

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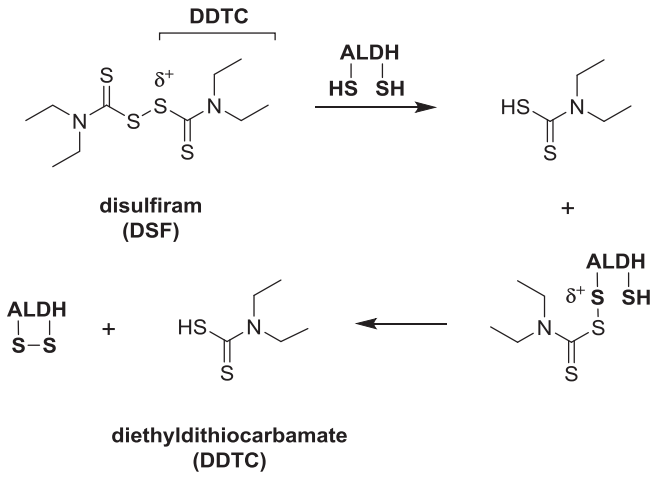
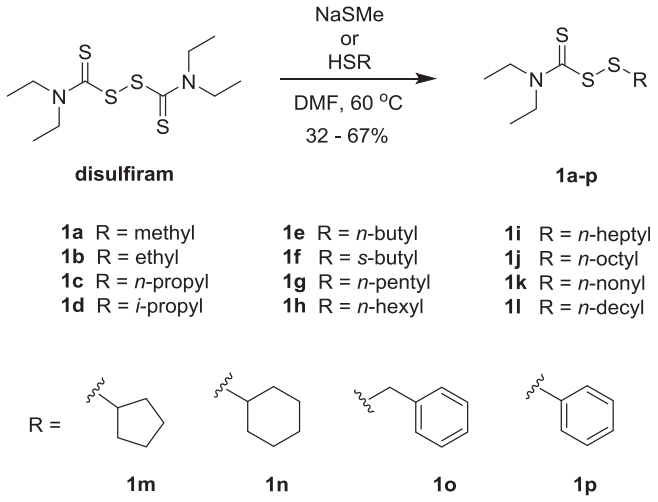


Fig. 1. Proposed route of *in vitro* aldehyde dehydrogenase (ALDH) inactivation by disulfiram.[2]



Scheme 1. Synthesis of disulfides **1** from disulfiram via a thiol-disulfide exchange reaction.

exhibited the greatest overall susceptibility to the DSF analogs followed by group A *Streptococcus pyogenes* (GAS), VAN-resistant *Enterococcus faecium* (VRE), *Streptococcus pneumoniae* (SP), and group B *Streptococcus agalactiae* (GBS). For VISA and VRSA variants of MRSA, the *S*-heptyl (**1i**) and *S*-octyl (**1j**) derivatives displayed equal or greater antibacterial activity than DSF and VAN.

The data in Table 1 further reveals a distinct correlation between the length of the *S*-alkylthio chain and antibacterial activity against Gram-positive cocci. Alkyl chains of seven (**1i**) and eight (**1j**) carbons were optimal lengths for antistaphylococcal activity with a MIC of 2–4 μM (0.6–1.2 μg/mL). By comparison, the MIC ranges of DSF and VAN were 8–64 μM (2.4–19 μg/mL) and 1–>32 μM (1.5–>48 μg/mL), respectively. Short straight chain analogs of one to five carbons were less active than their longer chain counterparts, but were more effective growth inhibitors of MRSA than DSF. Branch and cyclic carbon chain disulfides **1d**, **1f**, **1m**, and **1n** similarly had lower activity compared to the straight chain analogs and their respective unbranched equivalents **1c**, **1e**, **1g**, and **1h**.

Additional antibacterial testing of the compounds included the select agents *Bacillus anthracis* (anthrax), *Francisella tularensis* (tularemia) and *Yersinia pestis* (plague). Gram-positive *B. anthracis* exhibited the highest sensitivity to the DSF analogs followed by Gram-negative *F. tularensis* and *Y. pestis* (Table 2). In *B. anthraces*, it was noteworthy that a definitive structure-activity relationship could not be established for analogs with alkyl chains of one to eight carbons in length as seen in *S. aureus*. Moreover, DSF exhibited greater overall activity for all *B. anthracis* strains, but not to comparator ciprofloxacin (CIP), which was also the superior test agent against *Y. pestis* and *F. tularensis*.

To further delineate the antibacterial activity spectrum, the compounds were tested on nineteen additional Gram-positive (n = 5) and Gram-negative (n = 14) species. Table 3 shows that the inhibitory activity was confined to Gram-positive bacteria with *Bacillus cereus* exhibiting the greatest susceptibility followed by another rod-shaped species, *Listeria monocytogenes*. Similar to *B. anthraces*, activity was not predicated on chain length in *B. cereus*; however, chain lengths of seven (**1i**) and eight (**1j**) carbons were the most effective inhibitors of *L. monocytogenes* as observed with *S. aureus*. *Micrococcus luteus* and *Rhodococcus erythropolis* were also moderately susceptible at a MIC range of 16–32 μM. Conversely, the Gram-negative species panel as a whole displayed negligible

Table 1
Susceptibility of Gram-positive cocci to disulfides **1**.

Test agent	Species[a]|MIC (μM)							
	MRSA	VISA	VRSA	VISE	GAS	GBS	SP	VRE
1a	32	8	16	32	32	>32	>32	>32
1b	16	8	16	32	32	>32	>32	>32
1c	16	8	16	32	32	>32	>32	>32
1d	>32	32	>32	>32	32	>32	>32	>32
1e	16	16	16	32	32	>32	>32	>32
1f	>32	>32	>32	>32	32	>32	>32	>32
1g	16	8	16	8	>32	>32	>32	>32
1h	16	4	8	8	>32	>32	>32	>32
1i	4	2	4	4	16	>32	32	8
1j	4	4	4	4	16	32	16	8
1k	8	8	16	4	8	32	8	8
1l	16	16	32	16	4	16	4	16
1m	32	16	32	32	32	>32	>32	>32
1n	32	16	32	32	16	>32	>32	>32
1o	8	2	8	8	>32	>32	>32	32
1p	32	16	16	16	>32	>32	>32	>32
Disulfiram	32	8	32	32	16	>32	>32	>32
Vancomycin	1	4	>32	8	≤0.5	≤0.5	≤0.5	>32

[a] Methicillin-resistant *Staphylococcus aureus* COL (MRSA); vancomycin-intermediate resistant *S. aureus* ADR-217 (VISA); vancomycin-resistant *S. aureus* HIP14300 (VRSA); vancomycin-intermediate *Staphylococcus epidermidis* NRS6 (VISE); group A *Streptococcus pyogenes* H293 (GAS); group B *Streptococcus agalactiae* SGBS005 (GBS); *Streptococcus pneumoniae* TCH8431 (SP); vancomycin-resistant *Enterococcus faecium* ATCC 700221 (VRE).