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Disulfiram-based disulfides as narrow-spectrum antibacterial agents

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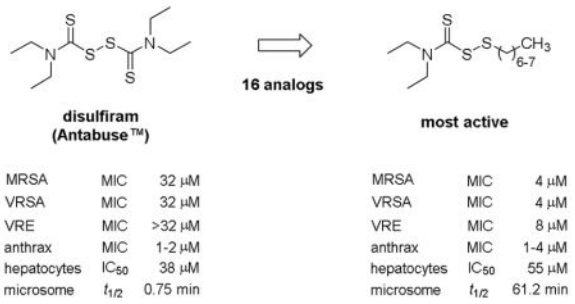
Abstract

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.

Graphical Abstract



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Keywords

disulfiram; disulfides; antibiotic; Staphylococcus; MRSA; VISA; VRSA

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 (Figure 1) [2]. As a versatile inhibitor of cysteine enzymes, DSF has been evaluated as a treatment for other clinical conditions. Recent U.S. clinical trials using repurposed DSF in treatment include: methamphetamine dependence (NCT00731133); cocaine addiction (NCT00395850); melanoma (NCT00256230); muscle atrophy in pancreatic cancer (NCT02671890); and HIV infection (NCT01286259) [5]. In the area of infectious disease, we recently established 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 (Figure 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* 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. anthraces* 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 susceptibility to the analogs. Based on the overall test results, it was concluded that DSF and analogs **1** possess similar narrow-spectrum profiles and straight chain derivatives of seven and eight carbons in length are the most potent growth inhibitors of Gram-positive cocci.

With a potential application as a treatment for resistant staphylococcal infections, analog **1j** was further evaluated for synergistic potential in comparison with DSF. The MICs of different VAN-**1j** and VAN-DSF concentration combinations were determined using the checkerboard microdilution assay in 96-well plate format [11,12]. Isobologram analysis revealed that analog **1j** and DSF lowered the MIC of VAN in MRSA, VISA, and VISE by comparable additive effects (Table 3) [13]. In VRSA, a synergistic effect was observed for both analog **1j** and DSF. From these studies it was concluded that disulfide **1j** and DSF can similarly lower the MIC of VAN and, therefore, both may have therapeutic utility as antibiotic adjuvants for staphylococcal infections with reduced VAN susceptibility.

The investigation also compared the cytotoxicity of the analogs with DSF in human liver HepG2 carcinoma cells using the MTT assay [14]. The dose-response curve in Figure 2 revealed that the half-maximal inhibitory concentrations (IC_{50}) of analogs **1c** (51 μM), **1h** (51 μM), **1j** (50 μM), and **1k** (55 μM) were above DSF (38 μM), thereby indicating lower cytotoxicity. The >13:1 ratio of *S. aureus* MIC (4 μM) to IC_{50} further suggests that compound **1j** is selectively toxic to the bacterium. The apparent selectivity was partially attributed to the higher glutathione content in mammalian cells [15]. The thiophilic tripeptide, which is found in low abundance in Gram-positive bacteria, has been shown to inactivate disulfide-based antibacterials through a thiol-disulfide exchange reaction [6,14,16].

Additional studies of analog **1j** included comparisons of microsomal stability and physiochemical properties to DSF (Table 4). Measurement of the *in vitro* metabolic stability using pooled rat liver microsomes indicated that the elimination half-life ($t_{½}$) and intrinsic clearance (CL_{int}) was longer for the DSF analog [17,18]. A comparison of the physiochemical chemical properties also revealed that both DSF and derivative **1j** are hydrophobic compounds. The higher clog*P* and lower molecular polar surface area (PSA) for disulfide **1j** suggest that replacement of the DDTC component in DSF with the more lipophilic *S*-octylthio group may confer better tissue and membranes penetration [19]. This marked difference with DSF could partially account for the increased susceptibility of *S. aureus* to analog **1j** if cell entry is required of both agents to inhibit growth.

In the final analysis, *S*-alkylthio analogs of DSF exhibited up to eight times greater antibacterial activity compared to DSF. The activity spectrum of the analogs was similar to DSF with Gram-positive *Staphylococcus* and *Bacillus* spp. exhibiting the highest level of susceptibility. Analogs with *S*-heptylthio (**1i**) and *S*-octylthio (**1j**) groups were found to be the most effective inhibitors of MRSA growth and retained their potency against VISA and VRSA. The select antibacterial activity was partly attributed to the low abundance of redox-buffering glutathione in the cytoplasm of Gram-positive bacteria [14]. Glutathione has been shown to inactivate disulfide-based antibacterial agents [6,14,16] and the higher abundance in Gram-negative bacteria may account for the Gram-type selectivity. This investigation also considered that the outer membrane barrier in Gram-negative bacteria could be a factor; however, the data from Table 3 indicated that increasing the lipophilic property of the compounds, which would facilitate cell membrane permeation, did not effect antibacterial activity in Gram-negative bacteria. Other factors that would account for the Gram-type selectivity, but were not investigated during the study, are the existence of potential pharmacological targets and cellular pathways required for antibacterial action.

This research further resolved that disulfide **1j** can lower the MIC of VAN in VISA and VISE, suggesting possible therapeutic utility as an antibiotic adjuvant in VAN-intermediate infections. Preliminary assessment of cytotoxicity and microsomal stability in comparison with DSF also confirmed that the *S*-octylthio analog was of lower toxicity to human hepatocytes and had a longer half-life, a parameter with implications on dosing frequency. Future research will focus on defining the mechanisms of action and resistance development for disulfide-based antibacterials. In addition, pharmacokinetic-pharmacodynamic (PK-PD) studies will be performed *in vivo* to determine their viability as antibiotic adjuvants in VAN

therapy. The PK studies will be used to guide dosing with a regimen that accounts for the influence of tissue glutathione on the disulfide concentration and gives a preferred trough VAN concentration of 4 to 5 times the MIC [22] with the disulfide for the test pathogen (e.g., VISA).

Acknowledgments

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References

1. Ellis PM, Dronsfield AT. Antabuse's diamond anniversary: still sparkling on? Drug Alcohol Rev. 2013; 32:342–344. [PubMed: 23194431]
2. Shen ML, Lipsky JJ, Naylor S. Role of disulfiram in the *in vitro* inhibition of rat liver mitochondrial aldehyde dehydrogenase. Biochem Pharmacol. 2000; 60:947–953. [PubMed: 10974203]
3. Shen ML, Johnson KL, Mays DC, Lipsky JJ, Naylor S. Determination of in vivo adducts of disulfiram with mitochondrial aldehyde dehydrogenase. Biochem Pharmacol. 2001; 61(5):537–45. [PubMed: 11239496]
4. Wright C, Moore RD. Disulfiram treatment of alcoholism. Am J Med. 1990; 88:647–655. [PubMed: 2189310]
5. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2018 Mar 2.
6. Long TE. Repurposing Thiram and Disulfiram as Antibacterial Agents for Multidrug-Resistant Staphylococcus aureus Infections. Antimicrob Agents Chemother. 2017; 61(9)
7. *Preparation of thiuram disulfide* ***1****; general procedure*: Disulfiram (200 mg, 0.68 mmol) and thiol (0.74 mmol) were combined in 1 mL of dry DMF and stirred for 10–18 h at 60°C. The solution was then cooled to room temperature, diluted with water and extracted twice with hexanes. The organic layers were combined, washed twice with water, dried over $MgSO_4$, filtered, and concentrated in vacuo Silica gel flash chromatography with 0–10% EtOAc in hexanes gave disulfide **1** as an oil. Spectra data of representative compound 1-[(diethylcarbamothioyl)disulfanyl]octane (**1j**): Yield 53%; pale oil; TLC (SiO_2) R_f 0.52 (9:1 hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07–4.02 (m, 2H), 3.84–3.78 (m, 2H), 2.84 (t, J = 7.2 Hz, 2H), 1.68–1.62 (m, 2H), 1.38–1.26 (m, 16H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.4, 51.8, 47.1, 38.8, 32, 29.3, 28.7, 22.9, 14.3, 13.3, 11.6; ESI–MS: *m/z* 294.2 $[M+H]^+$.
8. Cilibrasi V, Tsang K, Morelli M, Solfa F, Wiggins HL, Jones AT, Westwell AD. Synthesis of substituted carbamo(dithioperoxo)thioates as potential BCA2-inhibitory anticancer agents. Tetrahedron Lett. 2015; 56:2583–2585.
9. Jorgensen, JH., Turnidge, JD. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray, PR.Baron, EJ.Jorgensen, JH.Landry, ML., Pfaller, MA., editors. Manual of Clinical Microbiology. ASM Press; Washington, DC: 2007. p. 1152-1172.
10. *Susceptibility testing.* Bacteria were initially grown on Mueller-Hinton agar, Brucella agar, or tryptic soy agar with 5% sheep's blood from frozen stocks stored at −80°C. Test cultures were obtained from uniform colonies and enumerated overnight at 26 – 37°C ± 5% CO_2 in standard media: Mueller Hinton broth for *Bacillus* spp., *M. luteus* and *Staphylococcus* spp.; Todd Hewitt broth for *Streptococcus* spp.; tryptic soy broth for *Enterobacteriaceae* spp., *Burkholderia* spp. and *P. aeruginosa*; Brucella broth for *B. neotomae* and *C. freundii*; and brain heart infusion (BHI) broth for *E. faecium, F. tularensis*, *L. monocytogenes, P. multocida*, and *R. erythropolis*. All bacteria except, *R. erythropolis* (30°C) and *Yersinia* spp. (28 °C) were cultured at 37°C. *C. freundii*, *E. faecium*, *F. tularensis* and *Streptococcus* spp. were incubated under 5% CO_2 atmosphere. Briefly, overnight cultures adjusted to a 0.5 McFarland standard and diluted to 1:100 in the appropriate growth medium were treated with serial dilutions of disulfide **1** and disulfiram prepared as 1 mM or 1 mg/mL stocks in DMSO. Controls vancomycin and ciprofloxacin were prepared in ultrapure

pure water. The plates were then incubated for 20 hours and the MICs were recorded as the lowest drug concentration that gave complete inhibition of visual growth.

11. Moody, JA. Synergism testing: Broth microdilution checkboard and broth macrodilution methods. In: Isenberg, HD., editor. Clinical Procedures Handbook. ASM Press; Washington, DC: 1992. p. 5.18.1-5.18.28.
12. *Synergy testing*. Evaluation of synergistic activity was performed by isobologram analysis using the checkerboard microdilution assay. A 1:100 dilution of 0.5 McFarland bacterial suspension was used to inoculate a 96-well plate containing 2-fold serial dilutions of disulfide **1j** and VAN in 50 μL CAMHB. The plates were sealed with adhesive film and incubated for 20 h at 37°C. The fractional inhibitory concentration (FIC) index was calculated by dividing the MIC of the VAN-**1j** combination by the MIC of VAN or **1j** alone. The summative ΣFIC was calculated from the FIC values and interpreted according to the standard metrics: synergy ≤ 0.5; additive 0.5 < to ≤ 1; indifferent 1< to ≤ 4; antagonism > 4.
13. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother. 2003; 52:1. [PubMed: 12805255]
14. Sheppard JG, Mcaleer JP, Saralkar P, Geldenhuys WJ, Long TE. Allicin-inspired pyridyl disulfides as antimicrobial agents for multidrug-resistant Staphylococcus aureus. Eur J Med Chem. 2018; 143:1185–1195. [PubMed: 29126733]
15. Loi VV, Rossius M, Antelmann H. Redox regulation by reversible protein S-thiolation in bacteria. Front Microbiol. 2015; 6:187. [PubMed: 25852656]
16. Ramaraju P, Gergeres D, Turos E, Dickey S, Lim DV, Thomas J, Anderson B. Synthesis and antimicrobial activities of structurally novel S,S′-bis(heterosubstituted) disulfides. Bioorg Med Chem Lett. 2012; 22(11):3623–31. [PubMed: 22560472]
17. Shave, D., Alden, P. Application note. Waters Corporation; Milford, MA U.S.A: 2008. Determination of microsomal stability by UPLC/MS/MS.
18. *Microsome study*. Experimental solutions for the metabolic stability were prepared as per the method described in the Waters Corporation application note [15]. The metabolic reactions were initiated by adding the enzyme to the pre-incubated mixture of test compound and NADPH solutions in a 2 mL Eppendorf tube. The mixtures were incubated at 37°C for 0, 5, 10, 15, 30, and 60 mins with mild shaking. The reaction was terminated after each specified time point by placing it in an ice bath and adding 500 μl of cold MeCN. The incubation mixtures were then centrifuged for 10 mins at 3000 rpm and an aliquot of each supernatant transferred for analysis. Test compounds are quantified using LC/MS/MS, and its metabolic half-life in the *in vitro* test system is derived from percent remaining vs. time data. The LC-MS/MS setup consisting of Agilent 6490 triple quadrupole mass spectrometer equipped with an Agilent 1260 Infinity HPLC unit. Positive ESI LC-MS/MS was done using MRM transitions 297.06 > 214.8 and 294.14>148.1 for DSF and disulfide analog **1j**, respectively. Separation was performed on Agilent Eclipse plus C_{18} column eluted with MeOH-water gradient acidified with 0.1% formic acid.
19. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002; 45(12):2615–23. [PubMed: 12036371]
20. Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02.
21. Calculated from ChemDraw Ultra Software 8.0.
22. Hidayat LK, Hsu DI, Quist R, Shriner KA, Wong-beringer A. High-dose vancomycin therapy for methicillin-resistant Staphylococcus aureus infections: efficacy and toxicity. Arch Intern Med. 2006; 166(19):2138–44. [PubMed: 17060545]

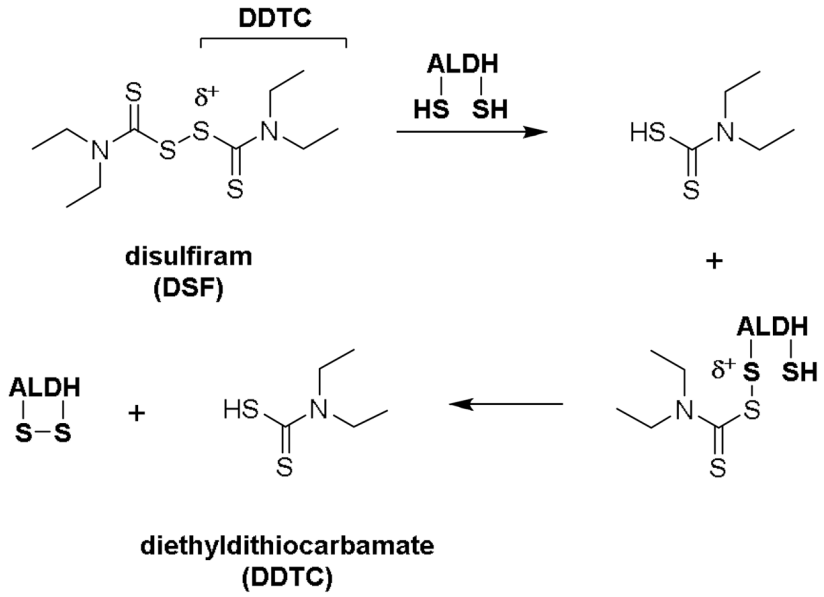


Figure 1.
Proposed route of *in vitro* aldehyde dehydrogenase (ALDH) inactivation by disulfiram [2].

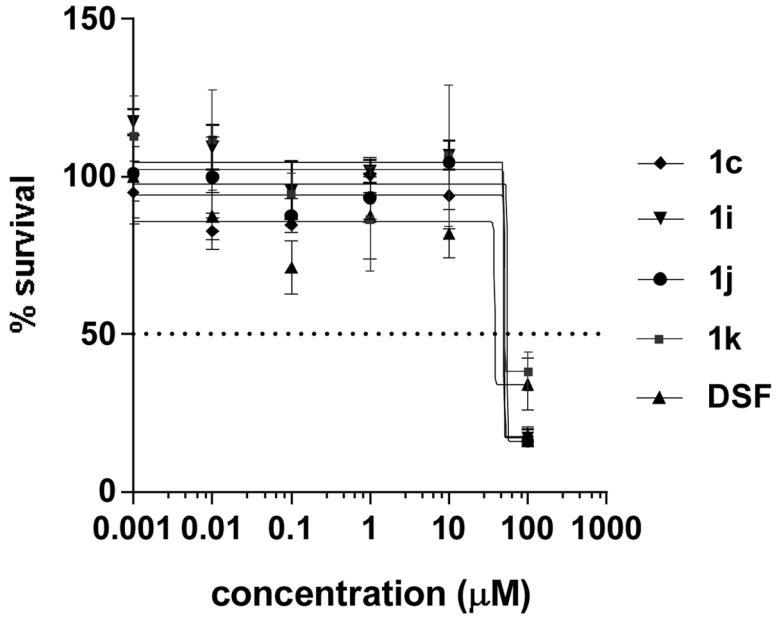
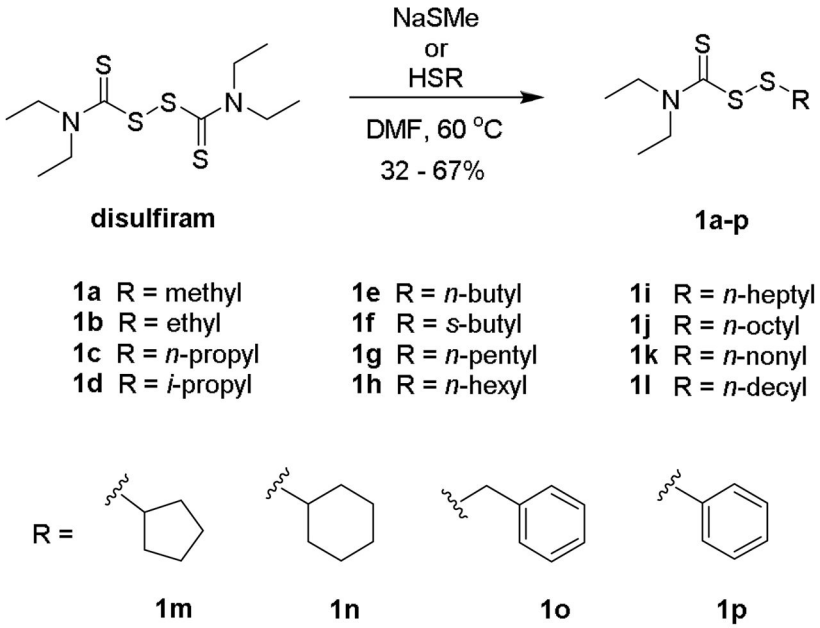


Figure 2.
Dose-response curve for liver HepG2 cells.



Scheme 1.

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

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).

Table 2

Antibacterial activity of disulfides **1**, disulfiram (DSF), and ciprofloxacin (CIP) against select agents.

species	strain	MIC (μM)								
		1a	**1c**	**1g**	**1h**	**1i**	**1j**	**1o**	**DSF**	**CIP**
Bacillus anthracis	Ames35	4	4	4	4	4	4	4	1	0.5
	Sterne 34F$_2$	4	4	4	4	2	2	4	2	≤0.5
	UM23-1	2	4	4	2	1	1	2	1	≤0.5
	Weybridge	4	4	8	8	2	2	4	2	0.5
Francisella tularensis	Utah 112	32	32	32	32	16	16	32	32	≤0.5
Yersinia pestis	Kim (D2)	16	16	32	32	32	32	16	32	≤0.5
	Kuma (D7)	16	32	32	32	32	32	32	32	≤0.5

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Table 3

Antibacterial activity of disulfides **1** against Gram-positive and Gram-negative species.

Gram-positive | -negative	strain	MIC (μM)								
		1a	**1c**	**1g**	**1h**	**1i**	**1j**	**1o**	**DSF**	**CIP**
Bacillus cereus	Gibson 971	1	1	1	2	1	1	2	4	0.5
Corynebacterium striatum	FS-1	>32	>32	32	16	16	16	32	>32	>32
Listeria monocytogenes	Gibson	32	16	8	8	4	4	8	>32	4
Micrococcus luteus	SK58	32	16	16	16	16	>32	16	32	4
Rhodococcus erythropolis	SK121	16	16	16	8	16	16	16	16	≤0.5
Acinetobacter baumannii	AB5075	>32	>32	>32	>32	>32	>32	>32	>32	≤0.5
Brucella neotomae	5K33	>32	>32	32	>32	>32	>32	32	>32	1
Burkholderia cepacia	UCB 717	>32	>32	>32	>32	>32	>32	>32	>32	4
Burkholderia multivorans	CF2	32	32	>32	>32	>32	>32	32	32	8
Citrobacter freundii	4_7_47CFAA	>32	>32	>32	>32	>32	>32	>32	>32	≤0.5
Escherichia coli	DC10B	>32	>32	>32	>32	>32	>32	>32	>32	≤0.5
Klebsiella pneumoniae	700603	>32	>32	>32	>32	>32	>32	>32	>32	2
Proteus mirabilis	HM-752	>32	>32	>32	>32	>32	>32	>32	>32	≤0.5
Pseudomonas aeruginosa	15442	>32	>32	>32	>32	>32	>32	>32	>32	1
Salmonella typhi	Ty2	>32	>32	>32	>32	>32	>32	>32	>32	≤0.5
Shigella dysenteriae	Newcastle 1934	>32	>32	>32	>32	>32	>32	>32	>32	4
Vibrio cholera	TS (D4)	32	32	>32	>32	>32	>32	>32	>32	≤0.5
Yersinia enterocolitica	WA-314	>32	>32	>32	>32	>32	>32	>32	>32	2
Yersinia pseudotuberculosis	P61	>32	>32	>32	>32	>32	>32	>32	>32	≤0.5

Table 3

Results of *in vitro* synergy studies.

strain	MIC (μg/mL)[a]					ΣFIC[d]	
	VAN	DSF	1j	VAN[b]/DSF[c]	VAN[b]/$_{1j}$	VAN[b]/DSF[c]	VAN[b]/$_{1j}$[c]
MRSA COL	2	8	2	1/1	1/0.25	0.63 (+)	0.75 (+)
VISA AR-217	4	4	0.5	2/1	0.5/0.25	0.75 (+)	0.56 (+)
VRSA HIP14300	>128	16	2	8/2	1/1	<0.16 (++)	0.5 (++)
VISE NRS53	8	4	2	4/2	2/1	1 (+)	0.75 (+)

[a] vancomycin: VAN; disulfiram: DSF

[b] lowest MIC of VAN in combination with DSF or **1j**

[c] lowest MIC of DSF or **1j** in combination with VAN

[d] lowest ΣFIC measurement; synergy (++) ≤ 0.5; additive (+) 0.5 < to ≤ 1; indifferent (±) 1< to ≤ 4; antagonism (−) > 4 [12]

Table 4

Microsomal stability and calculated physiochemical properties.

	DSF	1j
MW (g/mol)	296.5	293.6
p*K*a[a]	0.86	0.78
clog*P*[a]	3.82	6.53
PSA (Å^2)[a]	121	85.9
rotatable bonds[a]	7	11
$t_{½}$ (min)[b]	0.75	61.2
CL_{int} (μL/min/mg protein)[c]	1848	22.6

[a] calculated value [20,21]

[b] elimination half-life in pooled rat liver microsomes

[c] intrinsic body clearance