



6''-Thioether Tobramycin Analogues: Towards Selective Targeting of Bacterial Membranes**

Ido M. Herzog, Keith D. Green, Yifat Berkov-Zrihen, Mark Feldman, Roe R. Vidavski, Anat Eldar-Boock, Ronit Satchi-Fainaro, Avigdor Eldar, Sylvie Garneau-Tsodikova,* and Micha Fridman*

Decades of widespread clinical use of the bacterial ribosome A-site-targeting aminoglycosides (AGs) enhanced the evolution of resistance to these antibiotics and reduced their clinical efficacy.^[1] Three modes of action lead to bacterial resistance to AGs: reduction in the intracellular concentration of the antibiotics by efflux pump proteins or through reduced membrane permeability; structural modifications of the 16S ribosomal RNA that lead to reduced target affinity; and deactivation by AG-modifying enzymes (AMEs).^[1c,2] AMEs are divided into three families: AG nucleotidyltransferases (ANTs), AG phosphotransferases (APHs), and AG acetyltransferases (AACs).^[1b,3]

In many cases, AG-resistant bacteria have evolved combinations of resistance mechanisms, a fact that greatly increases the challenge of regaining their clinical efficacy through semisynthetic modifications. In recent years, several studies demonstrated the potential of exploiting AGs for the development of cationic amphiphilic antimicrobial agents by converting part or all of their pseudo-oligosaccharide alcohols into alkyl or aryl ethers.^[4] Some of these amphiphilic analogues demonstrated improved activities against several bacterial strains with resistance to the parent AG antibiotics. In addition to AG-based amphiphiles, several families of cationic amphiphiles including cationic steroids (ceragenins)^[5] as well as cationic antimicrobial peptides and peptidomimetic compounds^[6] have been developed and were found to possess potent antimicrobial activity. Unlike most mammalian cell membranes, bacterial membranes are rich in negatively-charged lipids, such as cardiolipins and phosphatidylglycerol, which attract cationic amphiphiles through ionic interactions,^[5] a fact that may be utilized for selective targeting of bacterial membranes.

Herein we report the design, synthesis, and antibacterial activity of 18 cationic amphiphiles (**4a–r**) derived from tobramycin (TOB; Scheme 1 A), which is a clinically important AG antibiotic that is becoming increasingly compromised by bacterial resistance. We also provide evidence for the mode of action of these derivatives and for the structural requirements for targeting bacterial membranes compared to targeting membranes of red blood cells (RBCs).

We chose to modify the 6'' primary alcohol of TOB and focused on two groups of lipophilic substituents: 1) aliphatic moieties including linear alkyl chains ranging from 6 to 22 carbon atoms in length as well as branched and cyclic alkyls, and 2) substituted aryl rings. The five amino groups of TOB were protected by Boc groups and the 6'' primary alcohol was selectively converted to the corresponding *O*-trisyl leaving group to provide compound **2** as reported (Scheme 1 A).^[7] Compound **2** was then reacted with each of the 18 aliphatic and aromatic thiols resulting in the Boc-protected compounds **3a–r** in yields ranging from 57 to 94%. Removal of all Boc protecting groups in neat TFA gave the TFA salts of the 6''-thioether TOB derivatives **4a–r** with no need for further purification in yields ranging from 74 to 98%.

Compounds **4a–r** were screened for their antibacterial activity against 21 Gram-positive and Gram-negative bacterial strains, and their minimum inhibitory concentrations (MICs) were determined (Table 1). Amongst the Gram-positive bacteria were pathogenic strains such as methicillin-

[*] Dr. K. D. Green,^[†] Dr. S. Garneau-Tsodikova
Department of Medicinal Chemistry and the Life Sciences Institute
University of Michigan
210 Washtenaw Ave, Ann Arbor, MI 48109 (USA)
E-mail: sylviegt@umich.edu
Homepage: <http://www.lsi.umich.edu/facultyresearch/labs/garneau>

I. M. Herzog,^[†] Y. Berkov-Zrihen, Dr. M. Feldman, Dr. M. Fridman
School of Chemistry, Tel Aviv University
Tel Aviv 66978 (Israel)
E-mail: mfridman@post.tau.ac.il

Dr. R. R. Vidavski, Dr. A. Eldar
Department of Molecular Microbiology and Biotechnology
Faculty of Life Sciences, Tel Aviv University (Israel)

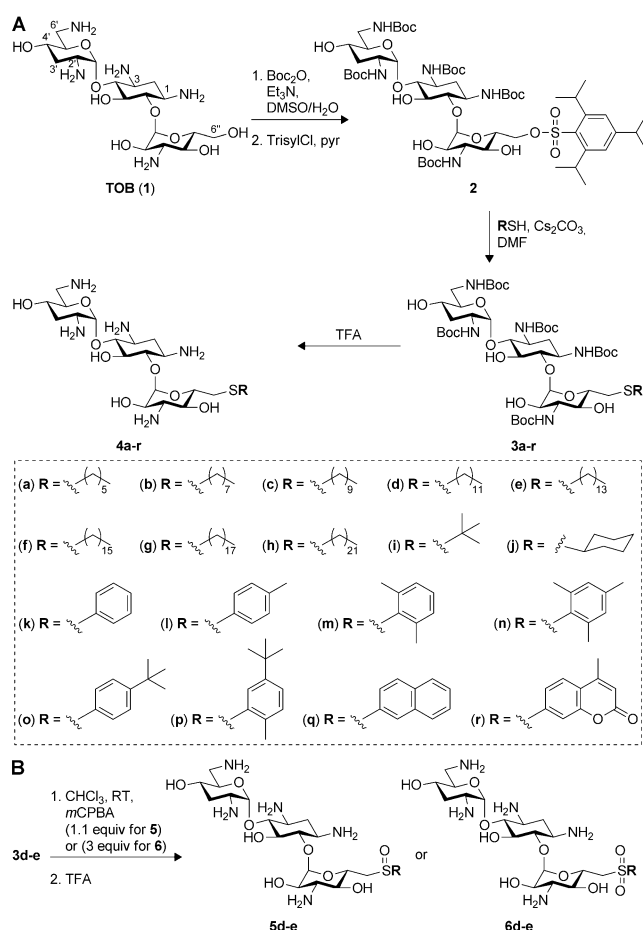
A. Eldar-Boock, Dr. R. Satchi-Fainaro
Department of Physiology and Pharmacology
Sackler School of Medicine, Tel Aviv University (Israel)

[†] These authors contributed equally to this work.

[**] This work was supported by the Life Sciences Institute and the College of Pharmacy at the University of Michigan (S.G.-T.), by a grant from the Firland Foundation (S.G.-T.), and by a National Institutes of Health (NIH) Grant AI090048 (S.G.-T.). This work was also supported by a grant from the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel (Grant 2008017, S.G.-T. and M.F.), and by the FP7-PEOPLE-2009-RG Marie Curie Auction: Reintegration Grants (Grant 246673, M.F.). We thank Profs. David H. Sherman and Philip C. Hanna (University of Michigan), Itzhak Ofek and Dani Cohen (Tel Aviv University), Doron Steinberg (The Hebrew University of Jerusalem), and Paul J. Hergenrother (University of Illinois at Urbana-Champaign) for the gift of bacterial strains used herein. We thank Prof. Timor Baasov and Dana Atia-Gilkin (Shulich Faculty of Chemistry, Technion, Israel Institute of Technology) for their assistance and guidance with the luciferase assay.



Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201200761>.



Scheme 1. A) Synthesis of 18 novel 6''-thioether TOB derivatives (**4a–r**). B) Oxidation of the thioether analogues **3d–e** into the corresponding sulfoxides **5d–e** and sulfones **6d–e**. Boc = *tert*-butoxycarbonyl, DMSO = dimethylsulfoxide, Trisyl = 2,4,6-triisopropylbenzenesulfonyl, pyr = pyridine, TFA = trifluoroacetic acid, mCPBA = *meta*-chloroperoxybenzoic acid.

resistant *Staphylococcus aureus* (MRSA; strain **C**) and vancomycin-resistant *Enterococcus* (VRE; strain **J**) that displayed high levels of resistance to TOB ($\text{MIC} \geq 150 \mu\text{g mL}^{-1}$). Amongst the Gram-negative were three strains of *E. coli* BL21 (DE3) that we cloned with AMEs: the bifunctional AAC(6')/APH(2''), AAC(3)-IV, and the multi-acetylating Eis, an AAC that confers high levels of resistance to AGs in extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (*Mtb*)^[8] (strains **O**, **P**, and **Q**, respectively). These three strains had significant to high levels of resistance to TOB ($\text{MICs} > 150, = 150$, and $18.8 \mu\text{g mL}^{-1}$, respectively).

The analogues with linear aliphatic chains **4a–h** exhibited a parabolic pattern of chain-length-dependent antibacterial activity (Figure S45A in the Supporting Information). Compared to the antibiotic TOB, both the C_6 and C_8 chain analogues **4a** and **4b** demonstrated a dramatic loss of antibacterial activity, the C_{10} chain derivative **4c** regained some activity, and the C_{12} chain analogue **4d** demonstrated potent antibacterial activity against several of the strains with resistance to TOB. The greatest improvement in antibacterial

activity was observed for the C_{14} and C_{16} chain derivatives **4e** and **4f**, with the most significant effect for **4e**, which showed marked activity against all of the 21 tested strains. The MIC values of **4e** ranged between 0.3 to $18.8 \mu\text{g mL}^{-1}$ against 19 of the 21 tested strains: the exceptions were *E. faecalis* (**K**) and *S. enterica* (**U**) where only a limited improvement in the antibacterial activity of **4e** as compared to TOB was observed (MIC of **4e**: 75 and $37.5 \mu\text{g mL}^{-1}$, respectively, and for TOB: $150 \mu\text{g mL}^{-1}$). The antibacterial activity dropped again for the C_{18} and the C_{22} chain analogues **4g** and **4h**. With few exceptions, a general drop in the antibacterial activity was observed for the 6'' aromatic thioethers (**4k–r**). The more substitution around the aryl ring, the more significant was the loss of antibacterial activity against the tested strains. For example, of the aromatic thioether analogues, **4k** with the thiophenyl ring, and **4l** with the 4-methyl-thiophenyl ring, demonstrated the best overall antibacterial activities against the tested strains. However, a drop in antibacterial activity was observed for the 2,6-dimethyl-thiophenyl derivative **4m**, and a more significant drop was observed for the 2,4,6-trimethyl-thiophenyl analogue **4n**. Since thioethers may be susceptible to cellular mediated S-oxidation,^[9] we oxidized two of the most potent thioethers (**4d–e**) to diastereomeric mixtures (ca. 4:1 ratio) of the corresponding sulfoxides (**5d–e**) and to the sulfones (**6d–e**; Scheme 1B). The effect of S-oxidation on the antibacterial activity varied amongst the tested bacterial strains. A reduction in the antibacterial activity of both sulfoxide and sulfone analogues as compared to the parent thioethers was observed for the *E. coli* BL21 (DE3) strains **M–Q** and for *B. subtilis* 168 with AAC(6')/APH(2'')-pRB374 (strain **G**). In contrast, when tested against *E. faecalis* (**K**) and *L. monocytogenes* (**L**), all four S-oxidized analogues demonstrated improved antibacterial activities compared to those of the thioethers **4d–e**. For most of the tested strains, S-oxidation did not have a dramatic effect on the antibacterial activity with MIC values identical or one double dilution higher than those of the thioethers **4d–e**. In most of the tested strains, MIC values were identical for the sulfoxides (**5d–e**) and the corresponding sulfones (**6d–e**), thus indicating that the level of S-oxidation has little to no effect on antibacterial activity.

To uncover the reasons for the broad spectrum and improved antimicrobial activity of some of the thioether analogues, we performed several biological tests. The effect of compound **4e** on the translation of a luciferase reporter gene was measured in *E. coli* cell lysates. In lysates of *Mycobacterium smegmatis* and *E. coli*, previous studies reported that the antibiotic TOB inhibited luciferase translation at IC_{50} values of approximately 20 nM .^[10] In our *E. coli* cell lysate, TOB potently inhibited translation ($\text{IC}_{50} = (8.9 \pm 1.9) \text{ nM}$), whereas **4e** did not reach an IC_{50} value even at 147 nM (measured using the free base forms of TOB and **4e**), thus suggesting that this compound does not target the bacterial ribosome as its major mode of antibacterial activity.

Furthermore, time-of-kill assays performed on *S. mutans* UA159 (**E**) and *S. pyogenes* (**D**) revealed that **4e** rapidly conferred bacterial cell death as compared to TOB (Figure S45B in the Supporting Information). At MIC values of $2.3 \mu\text{g mL}^{-1}$ for **4e** on both strains ($75 \mu\text{g mL}^{-1}$ (*S. mutans*) or

Table 1: Antibacterial activity: MIC values ($\mu\text{g mL}^{-1}$) of the 6''-thioether TOB derivatives **4a–r**, **5d–e**, and **6d–e** compared to the parent drug TOB.

Gram-positive bacterial strains ^[a]		Gram-negative bacterial strains ^[b]																				
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
TOB	0.3	9.4	>150	18.8	75	1.2	9.4	18.8	2.3	>150	150	4.7	4.7	9.4	>150	150	18.8	4.7	9.4	18.8	150	>150
4a	75	>150	>150	>150	>150	150	>150	>150	150	>150	>150	75	>150	>150	>150	>150	>150	>150	18.8	150	>150	>150
4b	75	150	>150	150	150	37.5	>150	150	75	>150	>150	>150	>150	>150	>150	>150	>150	>150	9.4	75	>150	>150
4c	9.4	37.5	75	150	9.4	37.5	>150	18.8	4.7	>150	>150	37.5	75	>150	>150	>150	>150	>150	2.3	9.4	>150	>150
4d	2.3	9.4	37.5	9.4	4.7	4.7	>150	4.7	9.4	>150	150	18.8	18.8	>150	>150	150	150	4.7	18.8	37.5	150	>150
4e	1.2	9.4	9.4	2.3	2.3	1.2	4.7	1.2	0.3	18.8	75	9.4	4.7	4.7	4.7	2.3	4.7	4.7	4.7	18.8	37.5	>150
4f	4.7	4.7	9.4	9.4	9.4	4.7	18.8	2.3	1.2	18.8	18.8	4.7	18.8	4.7	4.7	4.7	18.8	4.7	4.7	37.5	>150	>150
4g	9.4	>150	18.8	>150	>150	75	>150	>150	9.4	>150	150	75	>150	>150	>150	>150	>150	>150	4.7	>150	>150	>150
4h	75	>150	>150	>150	>150	150	>150	>150	18.8	>150	>150	18.8	>150	>150	>150	>150	>150	>150	>150	>150	>150	>150
4i	1.2	150	>150	75	75	75	>150	>150	75	>150	150	2.3	18.8	>150	>150	>150	150	18.8	9.4	150	>150	>150
4j	4.7	18.8	>150	37.5	>150	9.4	150	150	18.8	>150	150	4.7	75	>150	>150	>150	75	9.4	18.8	>150	>150	>150
4k	2.3	9.4	>150	18.8	>150	9.4	18.8	>150	9.4	>150	75	4.7	9.4	37.5	>150	>150	75	2.3	4.7	37.5	>150	>150
4l	4.7	9.4	>150	18.8	>150	4.7	9.4	150	9.4	>150	75	4.7	18.8	37.5	>150	>150	150	18.8	4.7	37.5	>150	>150
4m	18.8	75	150	>150	150	37.5	150	37.5	37.5	>150	150	9.4	75	150	>150	>150	150	4.7	18.8	>150	>150	>150
4n	75	150	>150	150	150	18.8	>150	75	18.8	>150	>150	37.5	150	>150	>150	>150	>150	9.4	150	>150	>150	>150
4o	75	>150	>150	150	150	>150	>150	>150	150	>150	>150	>150	>150	>150	>150	>150	>150	18.8	150	>150	>150	>150
4p	9.4	>150	>150	150	75	18.8	150	75	37.5	>150	>150	37.5	150	>150	150	>150	>150	9.4	37.5	>150	>150	>150
4q	18.8	37.5	>150	150	150	37.5	150	>150	37.5	>150	>150	37.5	75	150	>150	>150	>150	9.4	9.4	150	>150	>150
4r	37.5	>150	>150	>150	>150	>150	>150	>150	>150	>150	>150	150	>150	>150	>150	>150	>150	75	150	>150	>150	>150
5d	4.7	37.5	75	18.8	9.4	4.7	>150	9.4	2.3	150	18.8	9.4	75	>150	>150	>150	>150	2.3	18.8	150	>150	>150
5e	2.3	18.8	18.8	4.7	2.3	1.2	75	1.2	0.3	37.5	9.4	2.3	37.5	75	37.5	37.5	75	4.7	9.4	37.5	75	>150
6d	9.4	37.5	75	18.8	9.4	4.7	>150	9.4	2.3	75	9.4	9.4	75	>150	>150	>150	>150	2.3	18.8	>150	>150	>150
6e	2.3	18.8	18.8	4.7	4.7	1.2	37.5	1.2	0.3	37.5	4.7	2.3	18.8	75	37.5	75	75	2.3	9.4	37.5	75	>150

[a] *S. epidermidis* ATCC12228 (A), *S. aureus* NorA (B), MRSA (C), *S. pyogenes* serotype M12 (strain MGA59429) (D), *S. mutans* UA159 (E), *B. subtilis* 168 with AAC(6'')/APH(2'')-pRB374 (G), *B. cereus* ATCC11778 (H), *B. anthracis* 34F2 Sterne strain (I), VRE (J), *E. faecalis* ATCC29212 (K), and *L. monocytogenes* ATCC19115 (L). [b] *E. coli* BL21 (DE3) with pET22b (N), *E. coli* BL21 (DE3) with AAC(6'')/APH(2'')-pET22b (O), *E. coli* BL21 (DE3) with AAC(3)-IV-int-pET19b-pps (P), *E. coli* BL21 (DE3) with Eis (Q), *E. coli* ToIC (R), *E. coli* MC1061 (S), *Shigella* clinical isolate 6831 (T), and *S. enterica* ATCC14028 (U).

18.8 $\mu\text{g mL}^{-1}$ (*S. pyogenes*) for TOB) no viable bacteria remained in the sample treated with **4e** after five hours of incubation with *S. mutans* and three hours of incubation with *S. pyogenes*. For both of these strains, almost no reduction in bacterial growth was observed for TOB after the same incubation time. The results of the luciferase translation assay and the short time of kill displayed by **4e** suggested that this compound acts by disrupting the bacterial cell membrane. Additional strong evidence for the membrane disruption effect of **4e** was obtained by fluorescence microscopy experiments (Figure 1). Fluorescently labeled *B. subtilis* with constitutive YFP expression (PY79)^[11] was incubated for one hour with **4e** or with TOB at several concentrations. After one hour of incubation with TOB at both 2 $\times$ and 8 $\times$ the MIC (2.3 and 9.4 $\mu\text{g mL}^{-1}$, respectively), most of the bacterial cells in the sample were viable and maintained good fluorescence. In contrast, a significant drop in fluorescence, which presumably resulted from the bacterial cell lysis and loss of intracellular content including the YFP, was evident after the same incubation time with compound **4e** at both 2 $\times$ and 8 $\times$ the MIC (4.7 and 18.8 $\mu\text{g mL}^{-1}$, respectively).

The selectivity of the 6''-thioether derivatives **4b–h** towards bacterial membranes was tested by using a hemolysis assay on both laboratory rat and human RBCs (Figure 2). The MICs of the most potent thioether analogues ranged between 0.3 to 18.8 $\mu\text{g mL}^{-1}$. Hence, RBC samples were incubated with analogues **4d–f** at a concentration of 75 $\mu\text{g mL}^{-1}$, which is 4–250 times greater than the MIC range and at 18.8 $\mu\text{g mL}^{-1}$, which is 1–60 times the MIC range. At 75 $\mu\text{g mL}^{-1}$, TOB as well as compounds **4b** and **4c** with the linear C₈ and C₁₀ chains caused no measurable hemolysis of rat and human RBCs. Compound **4d** with the C₁₂ chain caused (12.6 $\pm$ 0.6)% hemolysis of rat RBCs and (7.9 $\pm$ 1.7)% hemolysis of human RBCs. Both compounds **4e** (C₁₄ chain) and **4f** (C₁₆ chain) caused extensive hemolysis at 75 $\mu\text{g mL}^{-1}$ with (93.6 $\pm$ 5.5)% and (90.2 $\pm$ 4.5)% of rat RBCs, and (93.7 $\pm$ 11.1)% and (93.1 $\pm$ 5.1)% of human RBCs, respectively. Compound **4g** (C₁₈ chain) caused (77.2 $\pm$ 7.0)% hemolysis of rat RBCs and (74.3 $\pm$

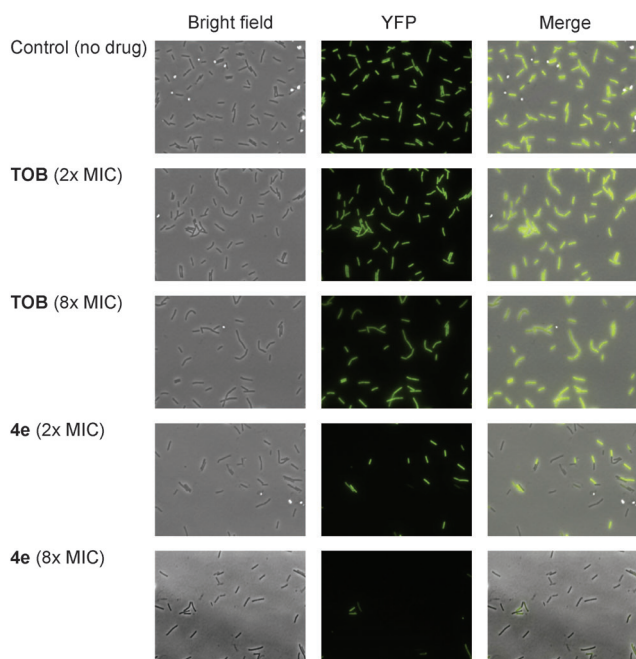


Figure 1. Bright-field and epi-fluorescence microscopy. *B. subtilis* (PY79) cells carrying the gene for the yellow fluorescent protein (YFP) under an inducible isopropyl- β -D-thio-galactoside (IPTG) promoter were treated with TOB at $2.3 \mu\text{g mL}^{-1}$ (2 $\times$ MIC) and $9.4 \mu\text{g mL}^{-1}$ (8 $\times$ MIC) or with compound **4e** at $4.7 \mu\text{g mL}^{-1}$ (2 $\times$ MIC) and $18.8 \mu\text{g mL}^{-1}$ (8 $\times$ MIC).

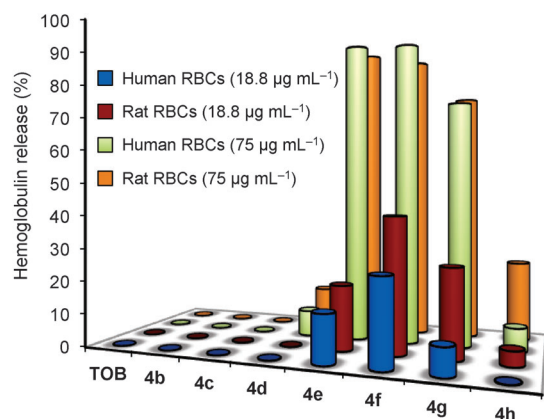


Figure 2. Hemolysis tests. Human RBCs and rat RBCs were incubated with TOB or with analogues **4b–h** at concentrations of $18.8 \mu\text{g mL}^{-1}$ or $75 \mu\text{g mL}^{-1}$ for 1 h at 37°C .

9.0%) hemolysis of human RBCs. A significant drop in the hemolytic activity was observed for compound **4h** (C_{22} chain), which caused hemolysis of (24.4 ± 5.8)% of rat RBCs and (7.1 ± 0.1)% of human RBCs.

At $18.8 \mu\text{g mL}^{-1}$, TOB and analogues **4b–d** with the linear C_8 , C_{10} , and C_{12} chains caused no measurable hemolysis of both rat and human RBCs, and compound **4e** (C_{14} chain) caused (19.5 ± 0.3)% hemolysis of rat RBCs and (14.3 ± 1.7)% hemolysis of human RBCs. Compound **4f** (C_{16} chain) demonstrated the maximal hemolytic effect of (40.4 ± 1.7)% (rat RBCs) and (25.4 ± 2.1)% (human RBCs), while **4g**

(C_{18} chain) caused (26.3 ± 1.9)% hemolysis of rat RBCs and (8.0 ± 0.8)% hemolysis of human RBCs. At $18.8 \mu\text{g mL}^{-1}$ compound **4h** (C_{22} chain) caused (4.4 ± 0.5)% hemolysis of rat RBCs and no measurable hemolysis of human RBCs. Although compound **4f** with the C_{16} chain was one of the most active TOB analogues against the tested bacterial strains, it readily disrupted RBC membranes as well. In contrast, compound **4d** (C_{12} chain) demonstrated potent antimicrobial activity against several of the TOB-resistant bacterial strains and caused little to no measurable hemolysis at the tested concentrations. The hemolysis assay demonstrated that the length of the aliphatic chain plays a key role in selective targeting of the bacterial membranes versus those of RBCs.

Several amphiphilic AGs based on paromomycin were recently shown to act as inhibitors of the AME APH(3').^[12] Moreover, amphiphilic AGs can make their way into the bacterial cell where they are exposed to enzymatic modifications by AMEs. Modifications by AMEs (O-phosphorylation, O-adenylation, and N-acetylation) result in a reduction of the overall positive charge of the parent AG. Hence, modifications by AMEs may reduce the affinity of amphiphilic AGs to the negatively-charged bacterial membrane and hamper their antimicrobial activity. Structural information obtained from crystallographic studies of several AMEs indicated that the AG binding pocket is rich in negatively-charged amino acid residues, such as glutamic and aspartic acids, and that several water molecules are required to stabilize the interactions between AGs and the AMEs' binding pockets.^[13] We reasoned that the replacement of the 6'' primary alcohol of TOB with hydrophobic residues would interfere with these hydrophilic binding interactions and reduce the ability of the enzymes to modify these molecules. To test this hypothesis, the relative activities of seven different AMEs^[14] with compounds **4a–r** as substrates were compared to that with TOB as substrate (Figure 3). While some of the modified compounds served as better substrates for some AMEs, in general a drop in the catalytic activity of the AMEs was observed in most of the cases. Analogues **4d–f**, which demonstrated the most potent antimicrobial activities, were also the poorest substrates for all of the tested AMEs.

Hence, bacterial strains that contain the tested AMEs will have limited to no ability to inactivate analogues **4d–f** through chemical modifications that are catalyzed by these enzymes. This inability is also true for the S-oxidized compounds **5d–e** and **6d–e**, which behaved similarly to their non-oxidized counterparts **4d–e** (Figure S46 in the Supporting Information).

In conclusion, 18 novel 6''-thioether TOB analogues (**4a–r**) and four S-oxidized compounds (**5d–e** and **6d–e**) have been synthesized and screened for antibacterial activity. The analogues **4d–f**, with linear C_{12} , C_{14} , and C_{16} chains, demonstrated potent activity against bacterial strains with high levels of resistance to TOB. We found evidence that the most potent analogue **4e** targets bacterial membranes and no longer targets the ribosome as does the parent drug. Hemolysis tests indicated that it is possible to improve the antibacterial activity while reducing the undesired hemolytic effect by altering the length of the aliphatic chain. Finally, thioethers

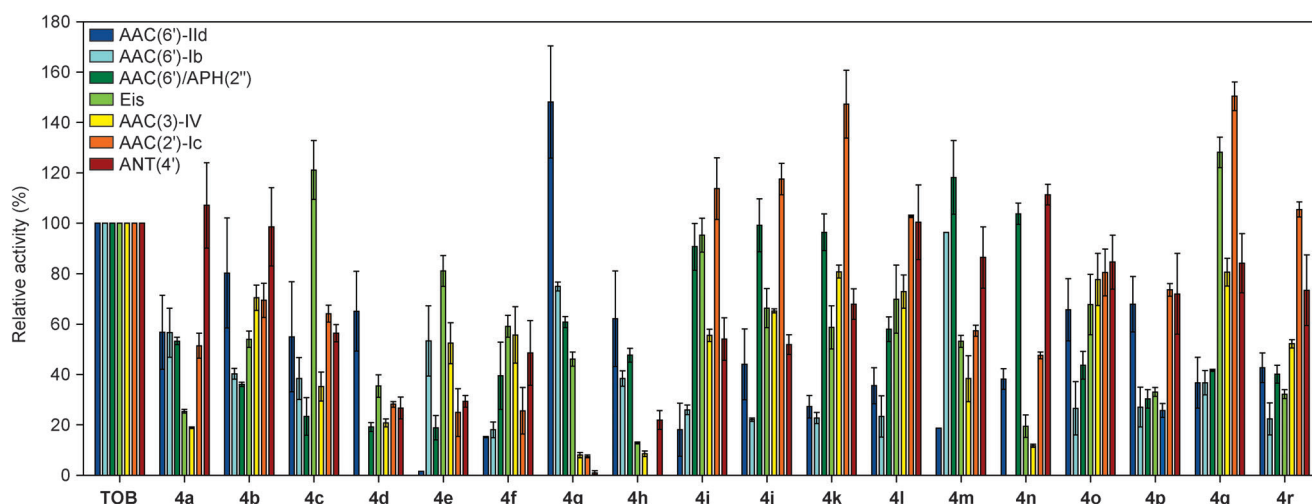


Figure 3. Bar graph showing the relative initial rates of reactions of the listed AMEs with TOB and the 6''-thioether analogues **4a–r**. Rates are normalized to TOB.

4d–f served as poor substrates for several AMEs, thereby demonstrating that AMEs have a limited deactivating effect on these AG analogues. The results reported herein offer guidelines for the design of amphiphilic AGs that target bacterial membranes.

Received: January 27, 2012

Revised: March 8, 2012

Published online: April 12, 2012

Keywords: aminoglycoside-modifying enzymes · amphiphiles · antibiotics · glycosides · hemolysis

- [1] a) M. Fridman, V. Belakhov, S. Yaron, T. Baasov, *Org. Lett.* **2003**, 5, 3575–3578; b) J. L. Houghton, K. D. Green, W. Chen, S. Garneau-Tsodikova, *ChemBioChem* **2010**, 11, 880–902; c) G. D. Wright, A. M. Berghuis, S. Mobashery, *Adv. Exp. Med. Biol.* **1998**, 456, 27–69.
- [2] a) S. Magnet, J. S. Blanchard, *Chem. Rev.* **2005**, 105, 477–498; b) M. P. Mingeot-Leclercq, Y. Glupczynski, P. M. Tulkens, *Antimicrob. Agents Chemother.* **1999**, 43, 727–737.
- [3] L. P. Kotra, J. Haddad, S. Mobashery, *Antimicrob. Agents Chemother.* **2000**, 44, 3249–3256.
- [4] a) M. Oubrai, F. El Garch, A. Bussiere, M. Riou, D. Alsteens, L. Lins, I. Baussanne, Y. F. Dufrene, R. Brasseur, J. L. Decout, M. P. Mingeot-Leclercq, *Biochim. Biophys. Acta Biomembr.* **2011**, 1808, 1716–1727; b) S. Bera, G. G. Zhanel, F. Schweizer, *J. Med. Chem.* **2010**, 53, 3626–3631; c) I. Baussanne, A. Bussiere, S. Halder, C. Ganem-Elbaz, M. Oubrai, M. Riou, J. M. Paris, E. Ennifar, M. P. Mingeot-Leclercq, J. L. Decout, *J. Med. Chem.* **2010**, 53, 119–127; d) S. Hanessian, K. Pachamuthu, J. Szychowski, A. Giguere, E. E. Swayze, M. T. Migawa, B. Francois, J. Kondo, E. Westhof, *Bioorg. Med. Chem. Lett.* **2010**, 20, 7097–7101.
- [5] R. F. Epand, P. B. Savage, R. M. Epand, *Biochim. Biophys. Acta Biomembr.* **2007**, 1768, 2500–2509.
- [6] a) B. Findlay, G. G. Zhanel, F. Schweizer, *Antimicrob. Agents Chemother.* **2010**, 54, 4049–4058; b) A. Giuliani, A. C. Rinaldi, *Cell. Mol. Life Sci.* **2011**, 68, 2255–2266; c) L. M. Yin, M. A. Edwards, J. Li, C. M. Yip, C. M. Deber, *J. Biol. Chem.* **2012**, 287, 7738–7745.
- [7] a) H. Wang, Y. Tor, *Bioorg. Med. Chem. Lett.* **1998**, 8, 3665–3670; b) H. Wang, Y. Tor, *Angew. Chem.* **1998**, 110, 117–120; *Angew. Chem. Int. Ed.* **1998**, 37, 109–111.
- [8] a) W. Chen, T. Biswas, V. R. Porter, O. V. Tsodikov, S. Garneau-Tsodikova, *Proc. Natl. Acad. Sci. USA* **2011**, 108, 9804–9808; b) K. D. Green, W. Chen, S. Garneau-Tsodikova, *ChemMedChem* **2012**, 7, 73–77.
- [9] a) M. A. Wynalda, J. M. Hutzler, M. D. Koets, T. Podoll, L. C. Wienkers, *Drug Metab. Dispos.* **2003**, 31, 878–887; b) A. G. Katopodis, H. A. Smith, S. W. May, *J. Am. Chem. Soc.* **1988**, 110, 897–899.
- [10] a) S. N. Hobbie, S. K. Kalapala, S. Akshay, C. Bruell, S. Schmidt, S. Dabow, A. Vasella, P. Sander, E. C. Bottger, *Nucleic Acids Res.* **2007**, 35, 6086–6093; b) S. J. Sucheck, A. L. Wong, K. M. Koeller, D. D. Boehr, K.-A. Draker, P. Sears, G. D. Wright, C.-H. Wong, *J. Am. Chem. Soc.* **2000**, 122, 5230–5231.
- [11] A. Eldar, V. K. Chary, P. Xenopoulos, M. E. Fontes, O. C. Loson, J. Dworkin, P. J. Piggot, M. B. Elowitz, *Nature* **2009**, 460, 510–514.
- [12] J. Szychowski, J. Kondo, O. Zahr, K. Auclair, E. Westhof, S. Hanessian, J. W. Keillor, *ChemMedChem* **2011**, 6, 1961–1966.
- [13] a) E. Wolf, A. Vassilev, Y. Makino, A. Sali, Y. Nakatani, S. K. Burley, *Cell* **1998**, 94, 439–449; b) L. E. Wybenga-Groot, K. Draker, G. D. Wright, A. M. Berghuis, *Structure* **1999**, 7, 497–507; c) J. Sakon, H. H. Liao, A. M. Kanikula, M. M. Benning, I. Rayment, H. M. Holden, *Biochemistry* **1993**, 32, 11977–11984; d) W. C. Hon, G. A. McKay, P. R. Thompson, R. M. Sweet, D. S. Yang, G. D. Wright, A. M. Berghuis, *Cell* **1997**, 89, 887–895.
- [14] a) K. D. Green, W. Chen, S. Garneau-Tsodikova, *Antimicrob. Agents Chemother.* **2011**, 55, 3207–3213; b) V. R. Porter, K. D. Green, O. E. Zolova, J. L. Houghton, S. Garneau-Tsodikova, *Biochem. Biophys. Res. Commun.* **2010**, 403, 85–90.