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# Oxa, Thia, Heterocycle, and Carborane Analogues of SQ109: Bacterial and Protozoal Cell Growth Inhibitors

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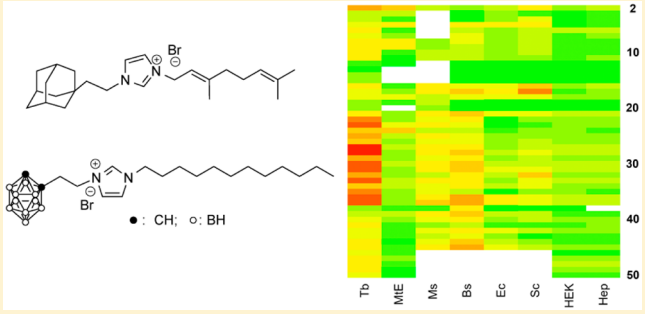
Supporting Information

**ABSTRACT:** We synthesized a library of 48 analogues of the *Mycobacterium tuberculosis* cell growth inhibitor SQ109 in which the ethylenediamine linker was replaced by oxa, thia, or heterocyclic species, and in some cases, the adamantyl group was replaced by a 1,2-carborane or the *N*-geranyl group by another hydrophobic species. Compounds were tested against *M. tuberculosis* (H37Rv and/or Erdman), *Mycobacterium smegmatis*, *Bacillus subtilis*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Trypanosoma brucei*, and two human cell lines (human embryonic kidney, HEK293T, and the hepatocellular carcinoma, HepG2). The most potent activity was found against *T. brucei*, the causative agent of human African trypanosomiasis, and involved targeting of the mitochondrial membrane potential with 15 SQ109 analogues being more active than was SQ109 in cell growth inhibition, having $IC_{50}$ values as low as 12 nM (5.5 ng/mL) and a selectivity index of ∼300.




**KEYWORDS:** *tuberculosis, sleeping sickness, uncouplers, menaquinone, trypanosomes*

The occurrence of drug resistance is a growing problem.[1,2] One serious threat is with tuberculosis because there are many millions of individuals infected with *Mycobacterium tuberculosis*, the causative agent of tuberculosis, resulting in ∼1.5 million deaths per year.[3] Chemotherapy is lengthy, and there is increasing resistance to antibiotics. New drugs and drug leads are thus needed. One of the oldest drugs for tuberculosis treatment is ethambutol (**1**), an ethylenediamine derivative, and in recent work some 74,000 analogues[4,5] of ethambutol including the ethylenediamine SQ109[4] (**2**) and the piperidine SQ609[5] have shown promise. One mechanism of action of SQ109 has been proposed to be inhibition of the membrane protein MmpL3,[6] a trehalose monomycolate transporter.[7] There have been no reports of spontaneous resistance to SQ109, but resistance to somewhat similar species involving MmpL3 has been reported,[8,9] and these *mmpL3* mutants have modest cross-resistance to SQ109.[6] SQ109 also has activity against other bacteria (e.g., *Helicobacter pylori*[10]) and fungi (e.g., *Candida abicans*[11]) as well as the malaria parasite *Plasmodium falciparum*, all of which lack the *mmpL3* gene, so in these organisms there must be other targets/mechanisms of action. SQ109 analogues might thus be of interest as anti-infective leads against a range of organisms. Here, we elected to synthesize four types of SQ109-inspired species that might have activity against bacteria, fungi, or protozoa.

We synthesized the SQ109 analogues (**3**−**50**) shown in Figures 1−3: (a) 13 alkanolamine analogues (**3**−**15**, Figure 1); (b) 3 thia analogues (**16**−**18**, Figure 1); (c) 23 heterocycle-containing analogues (**19**−**41**, Figure 2); and (d) 9 carborane-containing analogues (**42**−**50**, Figure 3). Full synthesis and characterization details are given in the Supporting Information.

In previous work, we found that compound **51** (Figure 1), the alkanolamine analogue of SQ109, was more active (0.035 μg/mL) against *M. tuberculosis* than was SQ109 (0.15 μg/mL).[12] We therefore first synthesized and tested 13 alkanolamine analogues of SQ109 (**3**−**15**, Figure 1) against *M.*

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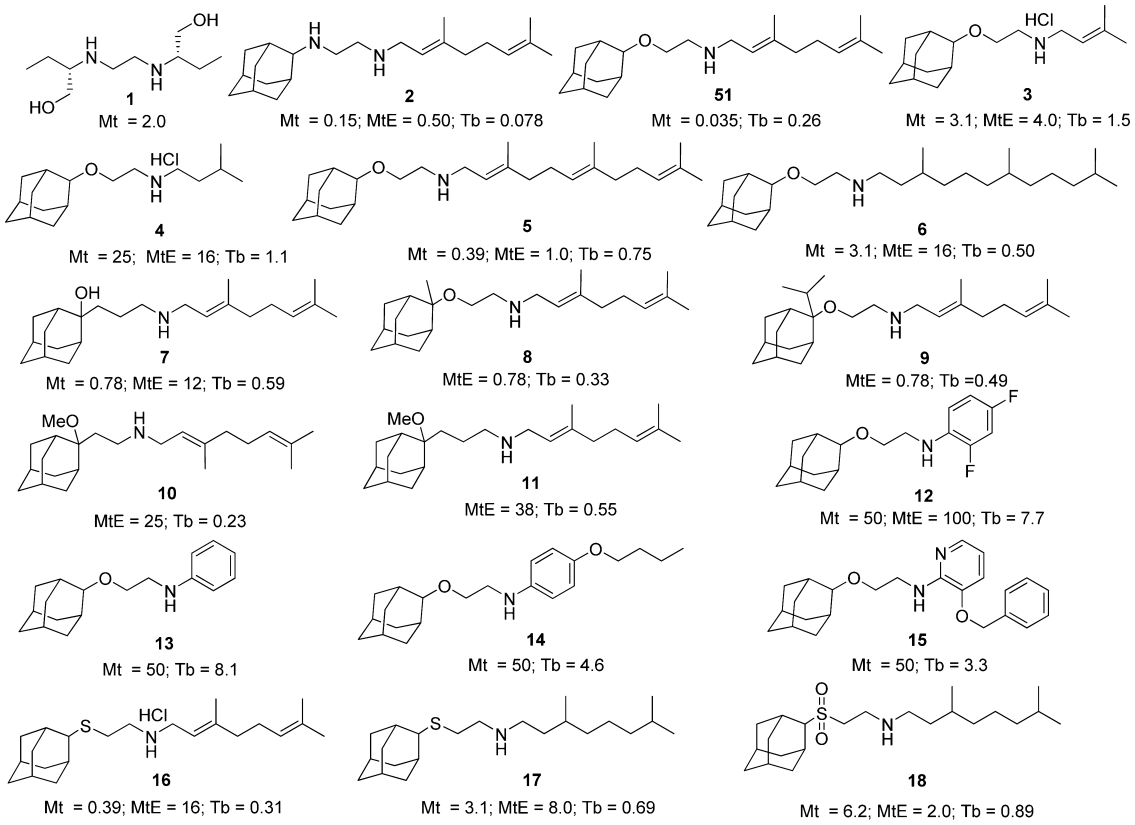


**Figure 1.** Alkanolamine and mercaptoethylamine analogues of SQ109 and their activities against *Mycobacterium tuberculosis* and *Trypanosoma brucei*. Mt, *M. tuberculosis* H37Rv; MtE, *M. tuberculosis* Erdman; Tb, *Trypanosoma brucei*. Values shown are in $\mu$g/mL and are MIC for the mycobacteria and $IC_{50}$ for *T. brucei*.

*tuberculosis*, *Mycobacterium smegmatis*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Escherichia coli*, and *Trypanosoma brucei* and HEK293T and HepG2 cells. MIC (*M. tuberculosis* H37Rv, *M. tuberculosis* Erdman), $IC_{50}$ (*M. smegmatis*, *B. subtilis*, *E. coli*, *S. cerevisiae*, *T. brucei*), and $CC_{50}$ (HEK293T and HepG2) values are given in Table 1 with the *M. tuberculosis* and *T. brucei* results shown, for convenience, below the structures in Figure 1.

There were several compounds with promising activity against *M. tuberculosis*. The most active compound was **5**, an analogue of SQ109 (**2**) in which the ethylenediamine nitrogen attached to the adamantane group was replaced by an oxygen and the geranyl ($C_{10}$) side chain by a farnesyl ($C_{15}$) group. The MIC was 0.39 $\mu$g/mL for *M. tuberculosis* H37Rv and 1.0 $\mu$g/mL for *M. tuberculosis* Erdman (MtE) (Figure 1; Table 1) to be compared with 0.1−0.5 $\mu$g/mL for SQ109 (**2**) in both strains and 0.035 $\mu$g/mL for **51** in *M. tuberculosis* H37Rv.[12] The reduced side-chain species **6** was ∼10−20 times less active than was the farnesyl analogue. The isopentenyl ethanolamine analogue (**3**) was also less active than was **5**, and reduction (**4**) reduced activity further. Incorporation of a 1-Me or 1 *i*-Pr group (**8**, **9**) decreased activity when compared with **51**. The presence of a 1-OH group (**7**) also resulted in decreased activity (0.78 $\mu$g/mL) over that found with SQ109. The O-methylated analogues (**10**, **11**) showed worse activity against MtE compared with the 1-OH species. Replacement of the isoprenoid side chains with aromatic groups (**12**−**15**) blocked all activity, and in other work[12] we found the diether analogue of **2** was also inactive.[12] These results indicate that optimum activity is found with a single nitrogen, that the order of activity of these alkanolamines is geranyl ≫ farnesyl ≫ isopentenyl, and that the reduced side-chain containing species are all less active than the unsaturated species. In the other assays (*B. subtilis*, *E. coli*, and *S. cerevisiae*) the most potent cell growth inhibitor (Table 1) was **5**, the *N*-farnesyl ethanolamine.

With the trypanosomatid parasite *T. brucei*, we found that SQ109 itself had quite potent activity against bloodstream form (BSF) parasites with an $IC_{50}$ of 0.078 $\mu$g/mL and a selectivity index (SI), defined as SI = $CC_{50}$ (HEK293T)/$IC_{50}$ (*T. brucei*) or $CC_{50}$ (HepG2)/$IC_{50}$ (*T. brucei*) in the 15−24 range (Table 1). The most active SQ109 analogues were **10** ($IC_{50}$ = 0.23 $\mu$g/mL), **8** ($IC_{50}$ = 0.33 $\mu$g/mL), and **6** ($IC_{50}$ = 0.50 $\mu$g/mL) with selectivity indices of 23, 21 (**10**), 19, 16 (**8**), and ∼3−4 (**6**), so these analogues are less promising than is SQ109 against *T. brucei*. We also tested the SQ109 analogue reported previously (**51**) to have potent activity against *M. tuberculosis*, but again it was slightly less active and had a worse SI as compared to SQ109 (Table 1).

We next investigated the three thia analogues of SQ109 (**16**−**18**) in which the N attached to adamantane in SQ109 (O in the more active ethanolamine analogue) was replaced by an S or $SO_2$ group (providing different H-bonding possibilities), and in two cases the geranyl group was reduced to the perhydro species. Cell growth inhibition results are shown in Table 1.

As can be seen in Figure 1 and Table 1, the thio-ether **16** had potent activity against *M. tuberculosis* H37Rv with an MIC of 0.39 $\mu$g/mL. **16** is the closest analogue to SQ109 in the compounds studied here and also had activity against *M. smegmatis* (1.2 $\mu$g/mL), *S. cerevisiae* (0.38 $\mu$g/mL), and *E. coli* (1.4 $\mu$g/mL). Interestingly, in these organisms, the reduced


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**Table 1. Growth Inhibition of Various Cells by SQ109 and Its Analogues[a]**

| | Tb[b] | Mt[c] | MtE[d] | Ms[e] | Bs[f] | Ec[g] | Sc[h] | HEK 293T[i] | HepG2[j] | SI (HEK 293T/Tb)[k] | SI (HepG2/Tb)[l] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ND | 2.0 | ND | 1.0 | ND | ND | ND | ND | ND | ND | ND |
| 2 | 0.078 ± 0.001 | 0.15 | 0.50 | 3.1 | 7.6 | 2.8 | 1.1 | 1.9 | 1.2 | 24 | 15 |
| 3 | 1.5 ± 0.1 | 3.1 | 4.0 | ND | >120 | 9.0 | 25 | 9.2 | 4.8 | 6.0 | 3.1 |
| 4 | 1.1 ± 0.1 | 25 | 16 | ND | >120 | 9.6 | 2.1 | 8.8 | 4.5 | 7.7 | 3.9 |
| 5 | 0.75 ± 0.1 | 0.39 | 1.0 | ND | 0.8 | 0.6 | 0.7 | 11 | 9.4 | 15 | 13 |
| 6 | 0.50 ± 0.1 | 3.1 | 16 | ND | 3.0 | 10 | 2.8 | 1.7 | 1.6 | 3.4 | 3.2 |
| 7 | 0.59 ± 0.05 | 0.78 | 12 | ND | 2.1 | 12 | 3.2 | 4.6 | 2.9 | 7.8 | 4.9 |
| 8 | 0.33 ± 0.06 | ND | 0.78 | 5.8 | 2.2 | 6.2 | 4.1 | 6.1 | 5.4 | 19 | 16 |
| 9 | 0.49 ± 0.09 | ND | 0.78 | 5.7 | 3.3 | 8.0 | 4.1 | 9.5 | 5.6 | 20 | 12 |
| 10 | 0.23 ± 0.03 | ND | 25 | 5.1 | 2.8 | 8.9 | 5.1 | 5.2 | 4.7 | 23 | 21 |
| 11 | 0.55 ± 0.1 | ND | 38 | 5.2 | 3.9 | 9.2 | 6.1 | 5.5 | 3.6 | 10 | 6.6 |
| 12 | 7.7 ± 1.5 | 50 | 100 | ND | >60 | >60 | >60 | 18 | 12.0 | 2.3 | 1.6 |
| 13 | 8.1 ± 0.1 | 50 | ND | ND | >60 | >60 | >60 | 16 | 7.3 | 2.0 | 0.9 |
| 14 | 4.6 ± 0.4 | 50 | ND | ND | >60 | >60 | >60 | 16 | 12 | 3.5 | 2.6 |
| 15 | 3.3 ± 0.5 | 25 | ND | ND | >60 | >60 | >60 | 21 | 20 | 6.4 | 6.1 |
| 16 | 0.31 ± 0.02 | 0.39 | 16 | 1.2 | 1.4 | 1.4 | 0.38 | 2.7 | 1.6 | 8.6 | 5.1 |
| 17 | 0.69 ± 0.12 | 3.1 | 8.0 | 1.1 | 0.5 | 0.7 | 0.1 | 4.9 | 4.1 | 7.1 | 6.0 |
| 18 | 0.89 ± 0.01 | 6.2 | 2.0 | 4.8 | 2.3 | 2.3 | 2.2 | 4.7 | 3.4 | 5.3 | 3.8 |
| 19 | 3.4 ± 0.8 | 12 | 25 | 4.6 | 28 | >74 | >74 | 17 | 19 | 5.0 | 5.6 |
| 20 | 2.8 ± 0.1 | 6.2 | ND | 5.6 | >90 | >90 | >90 | 12 | 12 | 4.2 | 4.2 |
| 21 | 0.39 ± 0.01 | 1.6 | 6.2 | 1.2 | 0.38 | 2.0 | 1.5 | 1.7 | 1.5 | 4.4 | 3.8 |
| 22 | 0.047 ± 0.007 | 3.1 | 12 | 1.5 | 1.2 | 36 | 9.1 | 3.2 | 4.3 | 68 | 91 |
| 23 | 0.023 ± 0.005 | 3.1 | 1.6 | 0.7 | 2.1 | 33 | 17 | 4.1 | 5.4 | 180 | 240 |
| 24 | 0.21 ± 0.02 | 0.78 | 0.50 | 3.2 | 3.1 | 13 | 8.4 | 4.9 | 6.2 | 24 | 30 |
| 25 | 0.058 ± 0.011 | ND | 3.1 | 1.2 | 2.1 | 12 | 5.7 | 2.6 | 3.1 | 45 | 53 |
| 26 | 0.31 ± 0.05 | ND | 6.2 | 1.5 | 2.0 | 12 | 4.8 | 5.1 | 5.5 | 17 | 18 |
| 27 | 0.0055 ± 0.0001 | ND | 3.1 | 0.9 | 0.88 | 7.2 | 4.4 | 1.6 | 2.0 | 290 | 370 |
| 28 | 0.0076 ± 0.0004 | ND | 6.2 | 0.9 | 0.41 | 5.4 | 2.2 | 1.3 | 1.5 | 170 | 200 |
| 29 | 0.12 ± 0.01 | ND | 6.2 | 1.6 | 0.78 | 5.6 | 3.8 | 3.7 | 3.7 | 30 | 30 |
| 30 | 0.019 ± 0.001 | ND | 4.6 | 1.0 | 0.48 | 3.4 | 2.7 | 1.6 | 1.9 | 86 | 100 |
| 31 | 0.017 ± 0.001 | ND | 6.2 | 1.0 | 0.37 | 2.0 | 1.6 | 1.6 | 1.5 | 92 | 86 |
| 32 | 0.12 ± 0.02 | ND | 9.3 | 3.5 | 0.56 | 3.6 | 0.51 | 4.4 | 4.2 | 38 | 36 |
| 33 | 0.016 ± 0.001 | ND | 4.6 | 0.8 | 0.59 | 6.7 | 1.9 | 1.8 | 2.1 | 110 | 130 |
| 34 | 0.15 ± 0.02 | ND | 3.1 | 1.8 | 0.69 | 4.0 | 2.1 | 3.2 | 3.5 | 21 | 23 |
| 35 | 0.022 ± 0.001 | ND | 12 | 2.3 | 3.3 | 35 | 12 | 3.2 | 5.4 | 97 | 160 |
| 36 | 0.021 ± 0.004 | ND | 6.2 | 0.8 | 0.22 | 1.0 | 1.4 | 1.5 | 1.5 | 70 | 70 |
| 37 | 0.026 ± 0.002 | ND | 12 | 0.5 | 0.24 | 1.0 | 1.8 | 1.9 | 1.6 | 73 | 61 |
| 38 | 3.0 ± 0.5 | ND | 17 | 16 | 1.2 | 85 | 46 | 19 | ND | 6.3 | ND |
| 39 | 1.4 ± 0.1 | ND | 3.1 | 1.1 | 0.36 | 4.6 | 4.5 | 5.2 | 6.3 | 3.8 | 4.7 |
| 40 | 0.93 ± 0.10 | ND | 6.2 | 1.9 | 1.1 | 6.4 | 3.5 | 3.6 | 3.4 | 3.9 | 3.6 |
| 41 | 0.40 ± 0.06 | ND | 25 | 7.3 | 3.8 | 19 | 16 | 6.3 | 4.9 | 16 | 12 |
| 42 | 0.68 ± 0.07 | ND | 25 | 1.3 | 0.75 | 2.1 | 3.4 | 3.5 | 3.0 | 5.1 | 4.4 |
| 43 | 0.93 ± 0.17 | ND | 25 | 3.8 | 1.6 | 11 | 29 | 7.2 | 5.1 | 7.7 | 5.5 |
| 44 | 0.63 ± 0.09 | ND | 37 | 1.9 | 0.31 | 5.0 | 7.3 | 7.9 | 7.8 | 13 | 12 |
| 45 | 0.49 ± 0.08 | ND | 25 | 0.8 | 0.2 | 1.8 | 3.0 | 4.1 | 3.7 | 8.4 | 7.6 |
| 46 | 0.96 ± 0.09 | ND | 25 | ND | ND | ND | ND | 14 | 14 | 15 | 15 |
| 47 | 0.37 ± 0.09 | ND | 12 | ND | ND | ND | ND | 2.5 | 3.1 | 6.8 | 8.4 |
| 48 | 0.33 ± 0.05 | ND | 25 | ND | ND | ND | ND | 1.9 | 1.4 | 5.8 | 4.3 |
| 49 | 0.61 ± 0.16 | ND | 100 | ND | ND | ND | ND | 4.7 | 4.2 | 7.6 | 6.8 |
| 50 | 1.2 ± 0.2 | ND | 100 | ND | ND | ND | ND | 7.5 | 7.9 | 6.4 | 6.7 |
| 51 | 0.26 ± 0.07 | 0.035 | ND | 1.6 | 16 | 2.8 | 1.8 | 1.3 | 1.0 | 4.9 | 3.8 |

[a]All units for MIC, $IC_{50}$ and $CC_{50}$ are μg/mL. The *T. brucei* results show mean and standard deviations of two independent experiments ($R^2$ for $pIC_{50}$ = 0.99); the fitting errors for Ms, Bs, Ec, and Sc obtained from dose−response curves (8 half-log dilutions) were 9, 9, 11, and 14%, respectively. *M. tuberculosis* inhibition MICs were estimated visually from 2× serial dilutions, while human cell growth inhibition was determined from fitting dose−response curves to a rectangular hyperbolic function. [b]Tb, *Trypanosoma brucei*, $IC_{50}$. [c]Mt, *M. tuberculosis* H37Rv, MIC. [d]MtE, *M. tuberculosis* Erdman, MIC. [e]Ms, *M. smegmatis*, $IC_{50}$. [f]Bs, *B. subtilis*, $IC_{50}$. [g]Ec, *E. coli*, $IC_{50}$. [h]Sc, *S. cerevisiae*, $IC_{50}$. [i]Human embryonic kidney, HEK293T, $CC_{50}$. [j]Human hepatocellular carcinoma, HepG2, $CC_{50}$. [k]SI = $CC_{50}$(HEK293T)/$IC_{50}$(Tb). [l]SI = $CC_{50}$(HepG2)/$IC_{50}$(Tb).

species was even more active (Table 1). The sulfone had weak activity in all assays. The results for *M. tuberculosis* are consistent with the results found for the alkanolamines **5** and **6** in that best activity is observed with the unsaturated side-chain-containing species. With *T. brucei*, the most active thia analogue was **16** ($IC_{50}$ = 0.31 μg/mL; SI = 5−9), followed by



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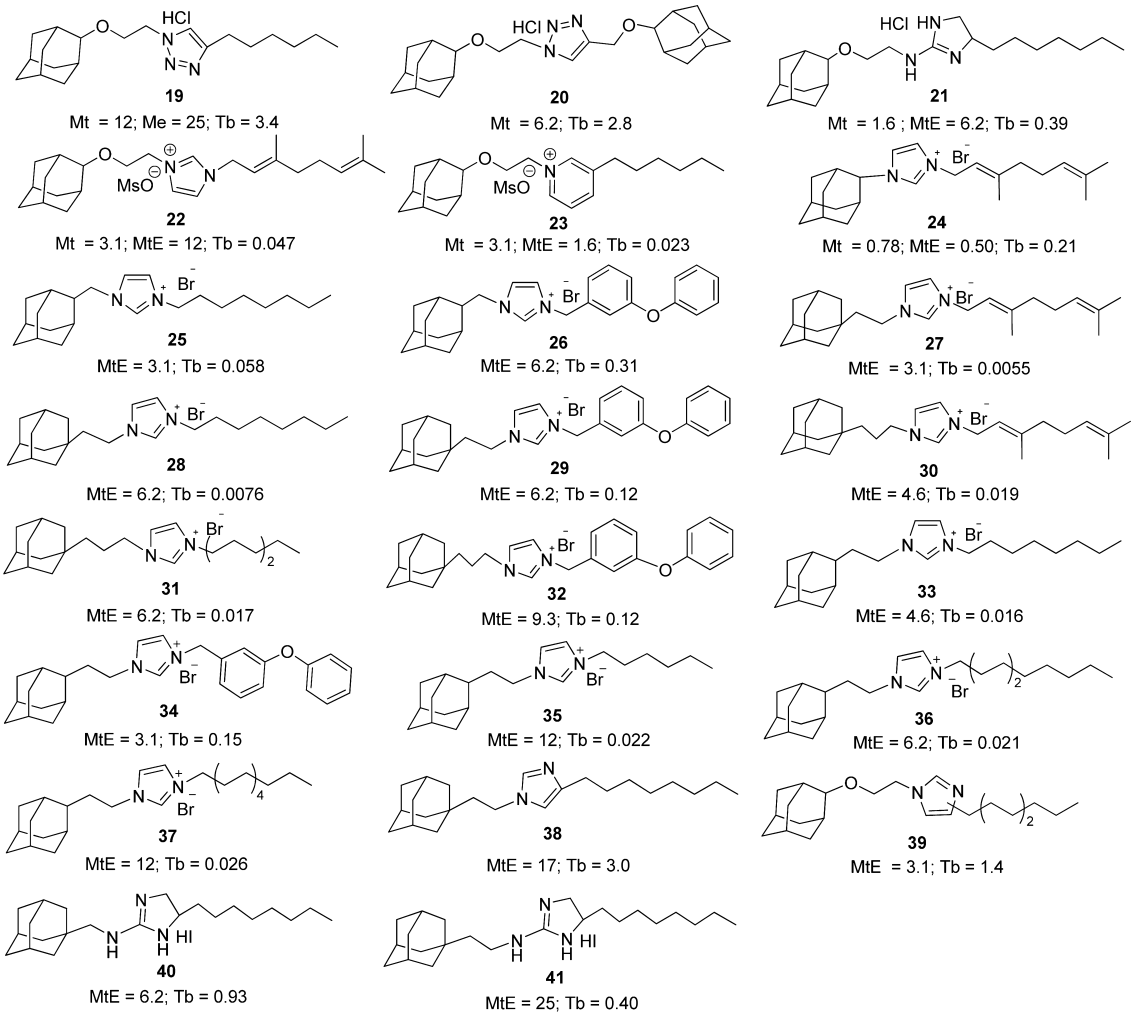


**Figure 2.** Heterocyclic analogues of SQ109 and their activities against *Mycobacterium tuberculosis* and *Trypanosoma brucei*. Mt, *M. tuberculosis* H37Rv; MtE, *M. tuberculosis* Erdman; Tb, *Trypanosoma brucei*. Values shown here are in $\mu$g/mL and are MIC for the mycobacteria and $IC_{50}$ for *T. brucei*.

**17** ($IC_{50}$ = 0.69 $\mu$g/mL; SI = 6−7) and **18** ($IC_{50}$ = 0.89 $\mu$g/mL, SI = 4−5).

The results described above are of interest in that we show, for the first time, that SQ109 has activity against the parasitic protozoan *T. brucei*, but unlike the situation found with the alkanolamine analogues of SQ109 reported previously,[12] none of the new analogues showed improved activity (over that seen with **51**) against *M. tuberculosis*, although **5**, **16**, and **17** were all more active than was SQ109 against the Gram-negative bacterium *E. coli* (**5**, $IC_{50}$ = 0.60 $\mu$g/mL; **16**, $IC_{50}$ = 1.4 $\mu$g/mL; **17**, $IC_{50}$ = 0.70 $\mu$g/mL, versus $IC_{50}$ = 2.8 $\mu$g/mL for SQ109; Table 1), although the computed selectivity indices (using HEK293T and HepG2) are poor (~5).

In previous work[12] we also found that another SQ109 analogue, a choline-derivative containing a quaternary ammonium instead of a protonatable N, had the most potent activity against a different parasitic protozoan, the malaria parasite *P. falciparum*, in addition to being a very potent inhibitor of respiration in *M. smegmatis*.[12] We thus reasoned that other cationic analogues of SQ109 might have better antibacterial and/or antiprotozoal activity, so we made and tested two further sets of analogues. We first synthesized a series of 23 SQ109 analogues with primarily protonatable (or fixed charge) heterocycle linker groups replacing the ethylenediamine fragment. The heterocycles investigated were neutral (the 1,2,3-triazoles **19** and **20**); protonatable (guanidines and amidines, **21**, **40**, and **41**; and an imidazole, **38**), or they contained a fixed positive charge (imidazoliums and pyridiniums, **23**−**39**). The two neutral triazoles had low activity against *M. tuberculosis* (**19**, MIC = 12, 25 $\mu$g/mL; **20**, MIC = 6.2 $\mu$g/mL) and *M. smegmatis* ($IC_{50}$ ~ 5 $\mu$g/mL) and essentially no activity against the other bacteria or the fungus.

Of the other heterocyclic compounds investigated, most had some activity against *M. tuberculosis* Erdman (and *M. tuberculosis* H37Rv) (Figure 2 and Table 1). However, there were only three compounds (**21**, **23**, and **24**) in which at least one of the *M. tuberculosis* MIC values was <2 $\mu$g/mL. Both **21** and **23** contain as a common structural feature the O−$CH_2$−$CH_2$−N group found in the potent alkanolamines, and in both cases, the nitrogen is expected to have either a formal +1 charge (**23**) or a large positive charge density (**21**, due to the strong basicity of the ligand and charge delocalization), so both resemble the protonated ethanolamines. In **24**, the aliphatic "linker" group is absent, but we now see that this potent inhibitor resembles SQ109 in another way in that it contains the N−C−C−N group found in the ethylenediamine fragment, which, in SQ109, is expected to carry a +1 charge (at pH ~7), again delocalized most likely over both nitrogens. As can be seen in Table 1, many of the other heterocyclic analogues have activity against the other bacteria as well as the fungus *S.*


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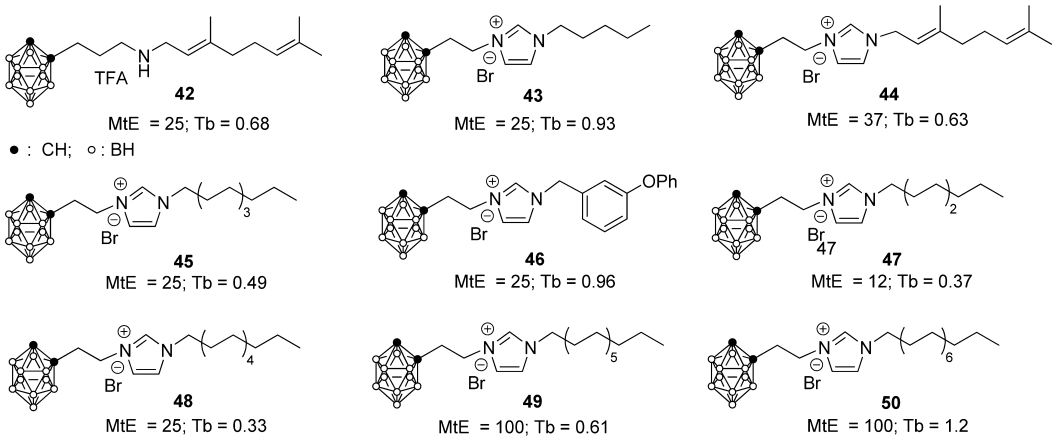


**Figure 3.** Carborane-containing analogues of SQ109 and their activities against *Mycobacterium tuberculosis* and *Trypanosoma brucei*. Mt, *M. tuberculosis* H37Rv; MtE, *M. tuberculosis* Erdman; Tb, *Trypanosoma brucei*. Values shown here are in $\mu$g/mL and are MIC for the mycobacteria and $IC_{50}$ for *T. brucei*.

*cerevisiae*, but they also inhibited the growth of the two human cell lines (Table 1), resulting in poor selectivity indices.

The results obtained against *T. brucei* were, however, much more encouraging (Table 1). Specifically, we found that there were 15 analogues of SQ109 that had better $IC_{50}$ and SI values than did SQ109 ($IC_{50}$ = 0.078 $\mu$g/mL; SI ∼ 15−24, Table 1). A typical set of dose−response curves for the top five *T. brucei* cell growth inhibitors, together with their corresponding effects on HEK293T and HepG2 cell growth, are shown in Figure S1, and selectivity index *versus T. brucei* cell growth inhibition results (for both human cell lines) are shown in Figure S2. The best *T. brucei* $IC_{50}$ value was 5.5 ng/mL, with corresponding SI values of 290 and 370 (Table 1). Clearly, these results are encouraging and, as noted above, are reminiscent of the activity of the choline analogue of SQ109 against *P. falciparum* in the intraerythrocytic assay where an $IC_{50}$ = 80 nM (35 ng/mL) was found (corresponding to SI ∼ 400);[12] plus, activity against the two human cell lines is similar to that seen with SQ109 (which is already in advanced clinical trials for tuberculosis).

Next, we sought to see whether improved activity might be found by replacing the adamantyl group by a 1-*o*-carboranyl group, which is similar to the adamantyl group in terms of size, shape, and hydrophobicity.[13] We produced the nine carboranes (**42**−**50**) shown in Figure 3. None had potent activity against *M. tuberculosis* Erdman (Table 1). However, in almost all cases there was activity against *M. smegmatis*, *B. subtilis*, *S. cerevisiae*, and, more surprisingly, against *E. coli*, with the ∼2 $\mu$g/mL $IC_{50}$ values found for **42** and **45** against *E. coli* being of interest because we found worse activity against this Gram-negative with the other analogues. Reasons for the enhanced activity against *E. coli* are unknown. Three compounds (**45**, **47**, and **48**) also had $IC_{50}$ < 0.5 $\mu$g/mL against *T. brucei*, although none approached the activity (and, hence, SI values) seen with the adamantane-containing analogues.

The most potent compound against *M. tuberculosis* Erdman is thus **24** with an MIC of 0.50 $\mu$g/mL, and **24** also has a 0.78 $\mu$g/mL MIC against *M. tuberculosis* H37Rv (Table 1). What is of interest about **24** is that it closely resembles the structure of SQ109 in that there are adamantyl and geranyl groups and a N−C−C−N linker but, here, the linker is an imidazolium, not an ethylenediamine group. The heterocycles (**19**−**41**) as a class have the most potent activity against the trypanosomatid parasite *T. brucei* and are also the most active against the two human cell lines. However, when the selectivity index values are calculated, it can be seen that **27** and **28** have the best $IC_{50}$ values of ∼5−7 ng/mL and SI ∼ 300. All of the compounds with the best SI (**22**−**37**) also have fixed charge centers, raising the question as to their possible mechanism of action.

In earlier work we found that SQ109 acted as an uncoupler in *E. coli* as well as in *M. smegmatis*, and we proposed that this uncoupling activity was important for its activity against *M. tuberculosis*.[12] Similar results have now been reported for a broader range of compounds that are now proposed to act as uncouplers in *M. tuberculosis*,[14] targeting ΔpH, Δ$\psi$, or both. We therefore tested SQ109 and the most interesting potential lead, **27**, in *T. brucei*, to see if similar effects were seen with either or both BSF and procyclic forms (PCF). We first tested whether SQ109 had effects on the proton motive force (more specifically, the inner mitochondrial membrane potential, Δ$\psi$) using the safranine method[15,16] with BSF parasites. Figure 4A shows that addition of 10 $\mu$M (3.3 $\mu$g/mL) SQ109 or 10 $\mu$M (4.5 $\mu$g/mL) **27** decreased Δ$\psi$, which was further reduced by addition of 8 $\mu$M (2 $\mu$g/mL) carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), a potent protonophore uncoupler. Similar results were obtained with PCF (Figure 4B). *T. brucei* mitochondria were able to phosphorylate ADP, as demonstrated by the small decrease in Δ$\psi$ after its addition (Figure 4C). This activity was inhibited by the ATP synthase inhibitor oligomycin. In addition, the mitochondria were able to transport $Ca^{2+}$, as shown by the decrease in the Δ$\psi$ after addition of $CaCl_2$, and the Δ$\psi$ returned to basal levels after addition of the $Ca^{2+}$-chelator EGTA. Further addition of SQ109 or **27** followed by FCCP again collapsed the Δ$\psi$ (Figure 4C). Both SQ109 and **27** collapsed Δ$\psi$ in a dose-dependent manner (Figure S3), and SQ109 alone or solvent (0.2% DMSO) had no effect. These results show that mitochondria in permeabilized *T. brucei* are able to develop a Δ$\psi$, phosphorylate ATP, and transport $Ca^{2+}$ and that SQ109 and **27** collapse Δ$\psi$. These effects on the proton motive force are rapid and are very similar to those observed for SQ109 in bacterial systems[17,18] and are likely to make a significant contribution to SQ109 and **27** inhibiting cell growth.

In addition to their effects on Δ$\psi$, it seemed possible that some compounds might act by inhibiting quinone biosynthesis, in some systems, just as other SQ109 analogues did with the prenyl transferase 1,4-dihydroxy-2-naphthoate octaprenyltrans-


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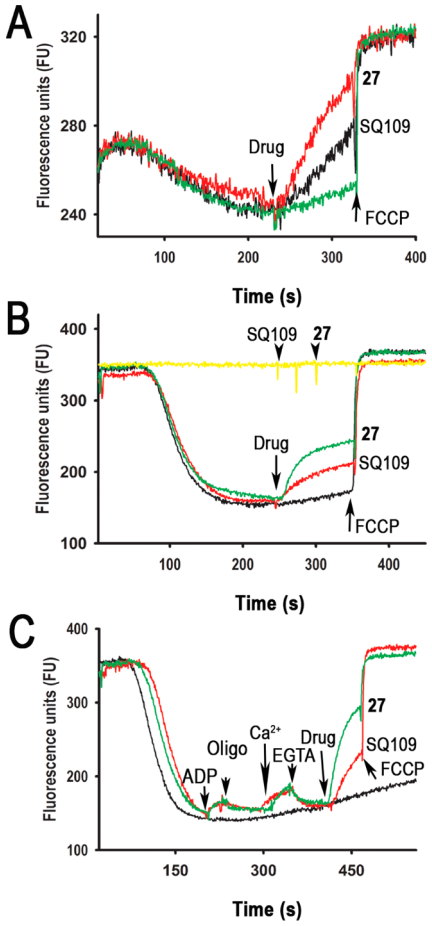


**Figure 4.** Effects of SQ109 or **27** on $\Delta\psi$ in digitonin-permeabilized *T. brucei*. (A) BSF trypanosomes ($2 \times 10^8$ cells) were added to reaction buffer (125 mM sucrose, 65 mM KCl, 10 mM Hepes–KOH buffer, pH 7.2, 1 mM $MgCl_2$, 2.5 mM potassium phosphate; 2 mL) containing 20 $\mu$M EGTA, 1 mM ATP, 500 $\mu$M orthovanadate, and 5 $\mu$M safranine, and the reaction was started with 40 $\mu$M digitonin. (B, C) *T. brucei* PCF ($5 \times 10^7$ cells) were added to the reaction buffer (2.4 mL) containing 2 mM succinate and 5 $\mu$M safranine, and the reaction was initiated with or without (yellow trace in B) 50 $\mu$M digitonin. SQ109 (3.3 $\mu$g/mL) and **27** (4.5 $\mu$g/mL) (equimolar amounts), FCCP (8 $\mu$M), ADP (10 $\mu$M), oligomycin (Oligo, 2 $\mu$g/mL), $CaCl_2$ (12 $\mu$M), and EGTA (200 $\mu$M) were added where indicated. No changes were detected in the absence of digitonin, indicating lack of secondary effects of the drugs.

ferase (MenA). We tested a representative set of compounds from the alkanolamine (**5**), imidazolium (**22**, **27**), imidazole (**39**), and carborane groups (**48**) against an expresssed *E. coli* MenA using the method reported previously.[12] Compounds **22** and **48** had no activity ($IC_{50}$ > 40 $\mu$M, 20 $\mu$g/mL), the $IC_{50}$ for **27** was 19 $\mu$M (8.5 $\mu$g/mL), and that for **5** was 9.0 $\mu$M (3.6 $\mu$g/mL), whereas that for **39** was 1.5 $\mu$M (0.54 $\mu$g/mL), suggesting that MenA inhibition with **39** could be of importance in MtE cell growth inhibition (MIC = 3.1 $\mu$g/mL). However, **39** has a poor SI.

Overall, the results reported above are of interest because we synthesized a broad range of analogues of the *M. tuberculosis* growth inhibitor, the ethylenediamine SQ109, and tested their activity against bacteria and a fungus, as well as a protozoan parasite. Protonatable or cationic species had the most activity, and the most potent leads against *M. tuberculosis* (MIC ~ 0.4–0.5 $\mu$g/mL) contained ethanolamine, mercaptoethylamine, or imidazolium linkers. The carboranes were less active against *M. tuberculosis* but, surprisingly, had activity ($IC_{50}$ ~ 2 $\mu$g/mL) against the Gram-negative *E. coli*. We did not obtain compounds that were more active against *M. tuberculosis* than was the ethanolamine analogue of SQ109 reported earlier. However, we did discover that the parent compound SQ109 had activity against the trypanosomatid parasite *T. brucei*, the causative agent of human African trypanosomiasis, and two SQ109 analogues had $IC_{50}$ values in the ~5–7 ng/mL range against this organism with SI values of ~300.

## METHODS

**Chemical Syntheses: General Methods.** All chemicals were of reagent grade. $^1$H NMR and $^{13}$C NMR spectra were obtained on Varian (Palo Alto, CA, USA) Unity spectrometers at 400 and 500 MHz for $^1$H and at 100 and 125 MHz for $^{13}$C. Elemental analyses were carried out in the University of Illinois Microanalysis Laboratory. HPLC-MS analyses were performed by using an Agilent LC/MSD Trap XCT Plus system (Agilent Technologies, Santa Clara, CA, USA) with an 1100 series HPLC system including a degasser, an autosampler, a binary pump, and a multiple-wavelength detector. All final compounds were ≥90% pure as determined by quantitative spin count NMR (qNMR, processed with Mnova NMR software), and structures were characterized by $^1$H NMR and HRMS. The synthesis and characterization of all new compounds (**3**–**50**) are shown in the Supporting Information.

***T. brucei* 427 (Bloodstream Forms) Growth Inhibition Assay.** *T. brucei* strain 427 bloodstream forms were cultivated at 37 °C with 5% $CO_2$ in HMI-9 medium supplemented with 10% fetal bovine serum (FBS). *T. brucei* parasites ($5 \times 10^4$/mL) were seeded in 384-well plates with or without a serial compound dilution. After 72 h of incubation, the parasites were exposed to 120 $\mu$M resazurin sodium salt (Sigma, St. Louis, MO, USA) and were incubated for another 5 h. Then, the parasites were fixed with 4% paraformaldehyde (PFA), and the assay plates were read by using a Victor 3 fluorometer (PerkinElmer, Waltham, MA, USA) at an excitation wavelength of 530 nm and emission of 590 nm. Pentamidine was used as a reference drug, and 0.5% DMSO was used as a drug-negative control. Two independent sets of experiments were carried out, and the mean and standard deviations are shown in Table 1; the $R^2$ for the $pIC_{50}$ correlation was 0.99.

## ASSOCIATED CONTENT

### Supporting Information

The following file is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.5b00026.

Full details of all assays; representative dose–response curves; selectivity index results; mitochondrial membrane bioenergetics; full synthesis and characterization of SQ-109 and its analogues as well as qNMR spectra (PDF)

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### Author Contributions

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### Author Contributions

K.L., Y.W., and E.O. designed the research; K.L., Y.W., and A.G. synthesized compounds; Y.W., G.Y., S.Y.B., G.R., C.S., D.C.C., M.C., and J.H.N. performed cell growth inhibition experiments;



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G.H. and R.D. performed mitochondrial membrane potential experiment; Y.W. and E.O. analyzed data; Y.W. and E.O. wrote the paper.

### Notes

The authors declare no competing financial interest.

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## ■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on April 9, 2015, with a minor error in the caption of Figure 4. The corrected version was reposted on April 10, 2015.



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