Synthesis and Testing of Analogs of the Tuberculosis Drug SQ109 Against Bacteria and Protozoa: Identification of Lead Compounds Against Mycobacterium abscessus and Malaria


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ABSTRACT

SQ109 is a tuberculosis drug candidate that has high potency against *Mycobacterium tuberculosis* and is thought to function at least in part by blocking cell wall biosynthesis by inhibiting the MmpL3 transporter. It also has activity against bacteria and protozoan parasites that lack MmpL3, where it can act as an uncoupler, targeting lipid membranes and Ca\(^{2+}\) homeostasis. Here, we synthesized 19 analogs of SQ109 and tested them against bacteria: *M. smegmatis*, *M. tuberculosis*, *M. abscessus*, *Bacillus subtilis* and *Escherichia coli*, as well as against the protozoan parasites, *Trypanosoma brucei*, *T. cruzi*, *Leishmania donovani*, *L. mexicana* and *Plasmodium falciparum*. Activity against the mycobacteria was generally less than with SQ109 and was reduced by increasing the size of the alkyl adduct, but two analogs were ~4-8 fold more active than was SQ109 against *M. abscessus*, including a highly drug resistant strain harboring a A309P mutation in MmpL3. There was also better activity than found with SQ109 with other bacteria and protozoa. Of particular interest, we found that the adamantyl C-2 ethyl, butyl, phenyl and benzyl analogs had 4-10x increased activity against *P. falciparum* asexual blood stages, together with low toxicity to a human HepG2 cell line, making them of interest as new anti-malarial drug leads. We also used surface plasmon resonance to investigate the binding of inhibitors to MmpL3, and differential scanning calorimetry to investigate binding to lipid membranes. There was no correlation between MmpL3 binding and *M. tuberculosis* or *M. smegmatis* cell activity, suggesting that MmpL3 is not a major target, in mycobacteria. However, some of the more active species decreased lipid phase transition temperatures, indicating increased accumulation in membranes, expected to lead to enhanced uncoupler activity.

INTRODUCTION

In 2020, an estimated 1.9 million people died from tuberculosis (TB), according to the World Health Organization. \(^1\) Treatment with combinations of antibiotics (isoniazid, rifampicin, pyrazinamide,
ethambutol) for six months can cure most people under optimal conditions, but globally, cure rates are less than optimal due to poor patient compliance to long-term chemotherapy, and the fact that some Mycobacterium tuberculosis (Mtb) strains have acquired mutations that render them resistant to many antibiotics. There is, therefore, the need for new, potent anti-tubercular drugs that have low rates of resistance. One such drug candidate is the N-geranyl-N’-(2-adamantyl)ethane-1,2-diamine SQ109, a second generation ethylenediamine which has been in phase II clinical trials and shows high potency against drug resistant Mtb. SQ109 binds to the trehalose monomycolate transporter, Mycobacterial membrane protein Large 3 (MmpL3), and is known to inhibit cell wall biosynthesis. MmpL3 transporter activity is driven by the proton motive force (PMF) and previous research has suggested that SQ109 can function by directly blocking the transporter’s pore or indirectly, by acting as an uncoupler. Importantly, SQ109 also has a growth inhibitory activity against other bacteria (e.g. Helicobacter pylori), fungi (e.g. Candida abicans), malaria parasites (e.g. Plasmodium falciparum), and the trypanosomatid parasite Trypanosoma cruzi, all of which lack the MmpL3 transporter, or at least close homologs. There is, therefore, interest in the synthesis and testing of SQ109 analogs since these might have enhanced activity against not only mycobacteria, but also against other bacteria as well as against protozoa, yeasts and fungi.

Here, we synthesized 19 analogs of SQ109 containing alkyl, aryl or heteroaryl groups at the C2-adamantyl position (Figure 1). Then, we tested SQ109 and the 21 analogs against the following mycobacteria: M. smegmatis, M. tuberculosis HN878, M. tuberculosis Erdman (MtErdman), M. tuberculosis H37Rv (MtH37Rv), and M. abscessus. In addition, we tested these analogs against Bacillus subtilis, Escherichia coli, Plasmodium falciparum asexual blood stages, human hepatocyte carcinoma cells, T. brucei bloodstream forms, T. cruzi epimastigotes and amastigotes and their U2OS host cells, L. donovani promastigote and L. mexicana promastigotes. We also explored the interaction of SQ109 and its analogs against MmpL3 using surface plasmon resonance (SPR), in addition to using differential scanning calorimetry (DSC) to explore the interactions of a subset of compounds with lipid bilayer membranes.

RESULTS AND DISCUSSION

Synthesis of SQ109 analogs. Based on the observation that the X-ray structure of SQ109 bound to MmpL3 has the adamantyl group located close to four aromatic rings, we selected to synthesize a series...
of analogs with substituents at the adamantyl C-2 position which we reasoned might have enhanced hydrophobic interactions with the protein, as well as with membranes. For the synthesis of compounds 8b-i, 12 we used as starting material geranylamine (2), 16,17 which we prepared from geraniol (1) using the Mitsunobu reaction (phthalimide, PPh₃, DEAD), and amines 5a-h, 9a, which we synthesized as described previously 18-20 (Figure 1). We carried out the preparation of the alcohol 5i from the reaction of the 2-thiazolyl lithium reagents (generated from 3 21, 22 with n-BuLi) and 2-adamantanone. We prepared the bromoacetamide (Figure 1) with Thz-Ph group 6i as well as 10, through a modified 20 Ritter reaction (BrCH₂CN, AcOH, H₂SO₄) from the corresponding tertiary alcohols 5i, 9b. 18-20 We prepared the bromoacetamide derivatives 6a-h and aminoacetamides 7b-c, 11 or 14b-h, 15 as previously described for SQ109 (8a). 23 In aminoamides 14a-h, 15 or 7a-i, 11, compared to SQ109 (8a), it seemed possible that the bulkier substituents at the adamantyl C-2 carbon might hinder reduction of the amide carbonyl, leading to a mixture of the desired ethylenediamines, accompanied by amine decomposition by-products, formed when LiAlH₄ in THF is used. 24-26 We thus synthesized the amide precursors 7a-i, 11 or 14a-h, 15 using LiAlH₄ in combination with freshly distilled trimethylchlorosilane (Me₃SiCl), in THF, with stirring for 2.5 h at 0-10 °C under an argon atmosphere, as described for SQ109 (8a). 23 We obtained the diamines 8a-i, 12 which were purified by using column chromatography, and were then converted to crystalline difumarate salts for cell growth inhibition testing. Aminoacetamides were tested as crystalline monofumarate salts.
Figure 1. Reagents and conditions: (a) Phthalimide, PPh₃, DEAD, and THF, rt, 24h (81%); (b) N₂H₄·H₂O, EtOH, reflux, 6h; (81%); (c) (i) n-BuLi, THF, -75 °C, 30 min; (ii) 2-adamantanone, THF, -75 °C, 1h; (iii) H₂O, 0 °C, (56%). (d) ClCOCH₂Br, K₂CO₃ (aq), CH₂Cl₂, rt, 24h (83-93%); (dii) BrCH₂CN, AcOH, H₂SO₄, rt, 1h (61%-quant.); (e) geranylamine (2), Et₃N, dry THF, rt, 48h (86-90%); (f) (i) Me₃SiCl, LiAlH₄, dry CH₂Cl₂, 0-5°C, Ar, 2.5 h; (ii) NaOH 10%, 0°C (20-40%).
**Anti-bacterial and antiprotozoal activity of SQ109 analogs.** We next investigated the activity of SQ109 (8a), its ethylenediamine analogs 8b-i, 12 and several aminoamide analogs: 7a, 7c, 7e, 7g, 7h, 14a, 14b, 14d, 11, 15 (Figure 1) against several bacteria, as well as protozoan parasites. We measured IC$_{50}$ or MIC values (Table 1) against the following bacteria: *M. smegmatis*, *M. tuberculosis* HN878 (a virulent clinical strain), MtErdman, MtbH37Rv, *M. abscessus*, *B. subtilis* and *E. coli*. The protozoa were *T. brucei* bloodstream forms, *T. cruzi* Dm28c epimastigotes and intracellular amastigotes (together with results for the U2OS host cells), *L. donovani* promastigotes, *L. mexicana* promastigotes and *P. falciparum* asexual bloodstream forms (ABS). For the protozoa, we determined parasite IC$_{50}$ and EC$_{50}$ values, as well as CC$_{50}$ values for host cells. Results are shown in Tables 2 and 3.

We first screened the 19 SQ109 analogs (together with SQ109) against *M. smegmatis* (Table 1). The IC$_{50}$ value for SQ109 (8a) against *M. smegmatis* was 2.4 μM, in good accord with previous work. Addition of an n-butyl group at C-2 (8e) yielded essentially the same result (IC$_{50}$ = 2.5 μM, Table 1) while shorter as well as more bulky substituents had less activity. For example, methyl (8b), ethyl (8c), n-propyl (8d), n-hexyl (8f), phenyl (8h) and benzyl (8g) group analogs had IC$_{50}$ values in the ~4-6 μM range. The larger substituted thiazole (8i) was even less active, with IC$_{50}$ = 20 μM (Table 1). Likewise, the C-1 substituted analog 12, containing a dimethylmethylene group, was less active, with an IC$_{50}$ value of 15 μM. Thus, the most active compounds have a relatively small substituent at C-2 and have IC$_{50}$ values in the ~2-4 μM range. We then investigated the activity of SQ109 and 17 of the SQ109 analogs against MtHN878, a hypervirulent *M. tuberculosis* strain. As can be seen in Table 1, the presence of a methyl group at C-2 (8b) yielded an IC$_{50}$ = 0.8 μM (Table 1) which is 2-fold higher than the IC$_{50}$ = 0.4 μM of SQ109. The larger alkyl adducts, e.g. ethyl (8c), n-propyl (8d), n-butyl (8e), n-hexyl (8f), benzyl (8g) and phenyl (8h) analogs showed comparable IC$_{50}$ values, in the ~1.6-3 μM range. In addition, we tested compounds against the *M. tuberculosis* Erdman and H37Rv strains, finding generally similar patterns of activity with the methyl adduct yielding an IC$_{50}$ = 2 μM (Table 1) and other analogs with alkyl groups having IC$_{50}$ values in the 4-8 μM range (Table 1). This activity was at least 2-fold lower than found with SQ109 (8a) (IC$_{50}$ = 1 μM), and the aminoamides (7a, 7c, 7e, 7g 7h, 14a, 14b, 14d, 11, 15) were generally less active than the more basic, ethylenediamine analogs.

In addition to investigating *M. tuberculosis*, we also investigated activity against *M. abscessus*, which is increasingly recognized as an emerging opportunistic pathogen causing severe lung disease, particularly in cystic fibrosis patients. Since it is intrinsically resistant to most conventional antibiotics, there is an unmet need for effective treatments. We determined the in vitro activity of SQ109 and a series of analogs against both smooth (S) and rough (R) variants of *M. abscessus*, which differ in their susceptibility profile to several antibiotics. As has been shown previously, the MIC of
SQ109 (8a) was much higher against *M. abscessus* (12.5-25 µg/mL) than against *M. tuberculosis* (~0.4-1 µg/mL). However, the n-butyl analog 8e as well as the benzyl analog 8g had significantly improved activity against both the S and R variants (MIC = 3.1 µg/ml versus 12.5-25 µg/mL), warranting future work on benzyl derivatives. The MIC values of all compounds against the R and S strains were either identical to or within a factor of 2x of each other. We also investigated an *M. abscessus* strain harboring an A309P mutation located in the transmembrane domain of MmpL3 (strain PIPD1R1), which displays very high resistance to the piperidinol-containing molecule PIPD1, 29 the indole-2-carboxamide Cpd12, 26 and the benzimidazole EJMCh-6. 26 Surprisingly, this mutant was not resistant to SQ109 or its analogs, having essentially the same MIC values as the wild type strains. This is a potentially important observation since resistance to other MmpL3 inhibitors can be very large (e.g. 32-64x for PIPD1). This lack of resistance may indicate that binding to MmpL3 is not the primary target of SQ109 (8a) and its analogs, in *M. abscessus*.

We then questioned whether any of the compounds investigated had activity against other bacteria, ones which lack MmpL3. In the gram-positive bacterium *B. subtilis*, SQ109 (8a) had an IC₅₀ value of 16 µM (Table 1) and of interest, several of the SQ109 analogs had much better activity. For example, n-butyl (8e), n-hexyl (8f) and phenyl (8h) analogs had IC₅₀ values in the 0.5 -1.0 µM range (Table 1). We also observed that some tested aminoamides had activity, in the 2-5 µM range. In the gram-negative bacterium *E. coli*, the IC₅₀ value for SQ109 (8a) was 15 µM and the n-alkyl (propyl, butyl, hexyl) analogs had IC₅₀ values in the ~ 8-10 µM range—slightly better than found with SQ109 (8a). Since neither *B. subtilis* nor *E. coli* contain the MmpL3 protein, other targets must be involved, so the promising activity of some compounds is of interest, for future work. There was either no inhibition or very low inhibition (IC₅₀ values > 70 µM) with the aminoamides tested (Table 1), suggesting that the ethylenediamine group might play a role in activity, as an uncoupler—as previously suggested for *Mtb* and protozoa.

Next, we investigated the activities of the SQ109 analogs against the following protozoa: *T. brucei*, *T. cruzi*, and two *Leishmania* species. In each case, the activity of SQ109 (8a) against these protozoa has been reported previously and has been proposed to arise from protonophore uncoupling, as well as effects on Ca²⁺ homeostasis. 15, 30, 31 Results for SQ109 (8a) and the analogs are shown in Table 2 and representative dose-response curves are shown in Figure S1. With *T. brucei* bloodstream forms, SQ109 (8a) had an IC₅₀ value of 0.91 µM and slightly better activity was found with the adamantanyl C-2 n-propyl (8d), n-butyl (8e), n-hexyl (8f) and benzyl (8g) analogs (IC₅₀ values of 0.88 µM, 0.37 µM, 0.98 µM and 0.85 µM, respectively), but is slightly less with phenyl (8h) and C-1 dimethylmethylene (12) analogs (IC₅₀ values of 1.3 µM, 1.4 µM, respectively). Of interest, the aminoamides also had
activity although IC$_{50}$ values were ~5-10-fold larger (in the range ~ 2-12 μM) compared to the ethylenediamines (8b-g, 12). With T. cruzi, we investigated both the epimastigotes as well as the (clinically relevant) amastigote forms, and activity against the host cell (U2OS). With epimastigotes, the IC$_{50}$ of SQ109 was 6.8 μM which is ~3-4x larger than that found with the C2 ethylene diamine analogs, n-propyl (8d), n-butyl (8e) and the C-1 dimethylmethylene analog (12). With aminoamide analogs, activity was again lower than with the ethylene diamine analogs. With the intracellular amastigote form, SQ109 (8a) had an IC$_{50}$ value of 0.74 μM and all of the ethylenediamine analogs had good activity, with the phenyl (8h) analog being comparable to (though not clearly better than) SQ109. The aminoamides were all less active, with IC$_{50}$ values in the range ~ 2-9 μM. The same trend in activity were seen with the inhibition of host cell growth, with the ethylenediamines being more potent host cell growth inhibitors than were the amides. With L. donovani, we tested SQ109 and 18 analogs (Table 2). The IC$_{50}$ value for SQ109 was 2 μM and the adamantyl C-2 methyl (8b), ethyl (8c), n-propyl (8d), n-butyl (8e), phenyl (8h), dimethylmethylene (12) analogs had higher or similar activity (in the 1-4 μM range), while the n-hexyl (8f) analog was less active (IC$_{50}$ = 8.8 μM) as was the benzyl (8g) analog (IC$_{50}$ = 6.1 μM). As with T. brucei and T. cruzi, the aminoamides were in general less (or much less) active with IC$_{50}$ values in the range ~ 5-44 μM. With L. mexicana the IC$_{50}$ value for SQ109 (8a) was 0.50 μM and the adamantyl C-2 methyl (8b) had an IC$_{50}$ = 0.43 μM while the n-propyl (8d) was ~4-fold less active (IC$_{50}$ = 1.94 μM). Several aminoamides had similar activity with 8d and amide 14d having IC$_{50}$ = 0.73 μM (Table 2).

In addition to investigating the trypanosomatid parasites, we also investigated the asexual bloodstream form of the apicomplexan parasite P. falciparum (PfABS). As previously reported, 26 SQ109 (8a) has activity against the PfABS parasite and only modest toxicity against HepG2 (human hepatocellular carcinoma) cells. We thus evaluated SQ109 and 13 of its analogs finding more potent (~100-900 nM) PfABS activity with the adamantyl C-2 methyl (8b), ethyl (8c), n-propyl (8d), n-butyl (8e), phenyl (8h) and benzyl (8g) analogs, as well as with the C-1 dimethylmethylene analog (12), compared to SQ109 (IC$_{50}$ = 1.6 μM, Table 3). The ethyl (8c) analog had ~10-fold better activity than that obtained with SQ109 (160 nM versus 1.6 μM), while the phenyl (8h) and benzyl (8g) analogs had 4x and 5x increased potency, respectively. Using HepG2 cell line viability as a test of overt toxicity, we found that there was 43% cell viability at 20 μM with the ethyl analog, but 100% viability with both phenyl and benzyl analogs, making them of potential interest as an antimalarial hits with low toxicity against mammalian cells.

When taken together, the results with M. tuberculosis cell growth inhibition indicate that it is difficult to improve upon the activity of SQ109 (8a) by adding substituents at the C-2 position. For
example, the methyl (8b) and benzyl (8g) substituents at the adamantyl C-2 group had ~2-4-fold less activity against MtbHN878, MtErdman and MtbH37Rv (Table 1). However, there was promising activity against both the S and R variants of M. abscessus. What is also of course of interest is the observation that some of the analogs do have better activity that does SQ109 against bacteria that lack the putative MmpL3 target, e.g. B. subtilis and E. coli, as also found with the malaria parasite, P. falciparum. A question then arises as to whether there is any correlation between MmpL3 binding and cell activity, in the mycobacteria.

Table 1. Anti-bacterial activity of SQ109 analogs

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<td>2.4</td>
<td>0.56</td>
<td>1.4</td>
<td>2.3</td>
<td>4.70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: Ms, M. smegmatis; Mtb, M. tuberculosis; Ma, M. abscessus; S, smooth variant; R, rough variant; Bs, Bacillus subtilis; Ec, E. coli. ND=not determined.

### Table 2. Activity of SQ109 analogs against Trypanosomatid parasites
<table>
<thead>
<tr>
<th>Comp. No</th>
<th>Chemical structure</th>
<th>PFABS IC₅₀ (µM ± S.E.)</th>
<th>HepG2 % viability @ 20 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>1.6 ± 0.22</td>
<td>97</td>
</tr>
<tr>
<td>8b</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>0.66 ± 0.18</td>
<td>100</td>
</tr>
<tr>
<td>8c</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>0.16 ± 0.067</td>
<td>43 (85% @ 2 µM)</td>
</tr>
<tr>
<td>8d</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>0.53 ± 0.22</td>
<td>100</td>
</tr>
<tr>
<td>8e</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>0.27 ± 0.042</td>
<td>50 (83% @ 2 µM)</td>
</tr>
<tr>
<td>8g</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>0.32 ± 0.032</td>
<td>100</td>
</tr>
<tr>
<td>8h</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>0.42 ± 0.091</td>
<td>100</td>
</tr>
<tr>
<td>7a</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>6.5 ± 0.33</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>7.6 ± 1.1</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>0.77 ± 0.098</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. Activity of SQ109 analogs against *Plasmodium falciparum* asexual blood stages
MmpL3–inhibitor binding interactions. We next used SPR to investigate the binding of SQ109 and several analogs to an expressed *M. tuberculosis* MmpL3 (MtMmpL3). We focused on measurement of the $K_D$ values for SQ109 (8a) and the 9 ethylenediamine analogs 8b-i, 12, together with four aminoamides 7a, 14a, 14b, and 14d. The experimental SPR results are shown in Figure S2. Data were fit using the two-state model, as described previously, together with the 1:1 binding model. The dissociation constants $K_D$ for both models are shown in Table 4. The two-state binding model gave slightly improved statistical fits but as can be seen in Table 4 there are essentially no differences between the $K_D$ values obtained using either model.

**Table 4. SPR results for MmpL3-ligand binding**

<table>
<thead>
<tr>
<th>Comp. No</th>
<th>Chemical structure</th>
<th>$K_D$, µM 2-state</th>
<th>$K_D$, µM 1:1 model</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>2060</td>
<td>2510</td>
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<td>8b</td>
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<td>8c</td>
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<td>12</td>
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<td>120</td>
<td>106</td>
</tr>
<tr>
<td>8d</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>106</td>
<td>91</td>
</tr>
<tr>
<td>8e</td>
<td><img src="image6" alt="Chemical structure" /></td>
<td>108</td>
<td>87</td>
</tr>
<tr>
<td>8f</td>
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<td>67</td>
</tr>
<tr>
<td>8g</td>
<td><img src="image8" alt="Chemical structure" /></td>
<td>74</td>
<td>69</td>
</tr>
<tr>
<td>8h</td>
<td><img src="image9" alt="Chemical structure" /></td>
<td>136</td>
<td>115</td>
</tr>
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</table>
What has been puzzling about the SPR result for SQ109 reported previously is that the $K_D$ value for SQ109 (8a) was high, ~1.5 mM, while the IC$_{50}$ against *M. tuberculosis* (IC$_{50}$ ≈ 0.4-1 μM, Table 1) is very low. The IC$_{50}$ for *M. smegmatis* is higher, ~2.4 μM, but again much lower that the $K_D$ value (Table 1). The $K_D$ values for all 14 compounds tested are lower than found with SQ109 (8a) (Table 4). What is of particular interest is that, compared to SQ109 (8a), there is a general decrease in the $K_D$ value, meaning tighter binding to MmpL3, as the compounds become more hydrophobic. For example, using the $K_D$ values from the two-state binding model that are shown in Table 4, with SQ109 (8a) and the adamantyl C-2 substituted species (see structure of R in Scheme 1) we found the following $K_D$ values R=H (8a or SQ109, 2060 μM); R=methyl (8b, 248 μM); R=ethyl (8c, 190 μM); R=n-propyl (8d, 106 μM); R=n-butyl (8e, 108 μM); R=n-hexyl (8f, 81 μM). That is, as the R substituent at C-2 (which is an H in SQ109) becomes larger and more hydrophobic, the $K_D$ decreased. A similar effect was observed with phenyl (8h, 136 μM) and benzyl (8g, 74 μM) C-2 substituents (Table 4). The C-1 dimethylmethylene analog 12 had a $K_D$ of 106 μM, which was close to the isomeric C-2 n-propyl 8d which had a $K_D$ of 120 μM. The thiazole 8i was also a relatively good binder with $K_D$ ~90 μM (Table 4).

As can be seen in Figure 2a, there is a significant correlation between the log $K_D$ values for all 14 compounds (i.e. including the four amides) and logD$_{7.4}$, the computed oil-water partition coefficient at pH=7.4, with a Pearson R coefficient R=0.73 and p<0.003. That is, the strongest binding occurs with the most hydrophobic species. This is not unexpected, however, we find no correlation between cell growth inhibition and log $K_D$, Figure 2b, or between cell growth inhibition and logD$_{7.4}$, for all of the compounds tested, Figure 2c. Essentially the same results were obtained using either the 1:1 or 2-state models for $K_D$. 

<table>
<thead>
<tr>
<th></th>
<th>Structure</th>
<th>$K_D$ (μM)</th>
<th>$R_D$ (μM)</th>
</tr>
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<tbody>
<tr>
<td>8i</td>
<td><img src="image" alt="Structure" /></td>
<td>91</td>
<td>87</td>
</tr>
<tr>
<td>7a</td>
<td><img src="image" alt="Structure" /></td>
<td>321</td>
<td>250</td>
</tr>
<tr>
<td>14a</td>
<td><img src="image" alt="Structure" /></td>
<td>280</td>
<td>644</td>
</tr>
<tr>
<td>14b</td>
<td><img src="image" alt="Structure" /></td>
<td>531</td>
<td>426</td>
</tr>
<tr>
<td>14d</td>
<td><img src="image" alt="Structure" /></td>
<td>267</td>
<td>252</td>
</tr>
</tbody>
</table>
Figure 2. Graphs showing correlations between log $K_D$, LogD$_{7.4}$ and $M. smegmatis$ cell growth inhibition. (a) Log $K_D$ versus logD$_{7.4}$; R=0.73, p=0.003. (b) Log Ms IC$_{50}$ and log $K_D$; R=0.06, p=0.843 (c) Log Ms IC$_{50}$ and logD$_{7.4}$, for all compounds tested; R=0.322, p=0.261. Selected compound numbers are shown.

One possible reason for this is that while the more hydrophobic species 8b-i, 12 do bind more strongly to MmpL3 compared to SQ109 (8a), there may be unfavorable steric interactions with the highly glycosylated mycobacterial cell wall with the larger substituents, or with any transporters that may facilitate cell entry. It is also of course possible that there may be other targets such as MenA, MenG, involved in menaquinone biosynthesis, which could contribute to the observed differences in activities. The activity of other proteins may also be targeted, together with binding to lipid membranes, affecting uncoupling activity and the proton motive force, as well as affecting lipid membrane inhibitor-concentrations (and thus, activity). The importance of steric effects in influencing poor cell uptake in the mycobacteria seems likely since as can be seen in Table 1 and Figure 2, the bulky adamantyl C-2 phenylthiazoyl (8i) analog—while exhibiting stronger binding to MmpL3 than does SQ109 (8a) —had only very weak activity against $M. smegmatis$ cell growth. With $M. tuberculosis$ HN878, where full dose-response curves were obtained, there was a similar trend, with SQ109 (8a) being most active (IC$_{50}$ = 0.4 μM), followed by the C-2 Me analog 8b (IC$_{50}$ = 0.8 μM), the ethyl analog 8c (IC$_{50}$ = 1.6 μM), n-propyl (8d) and n-hexyl (8f) analogs both having IC$_{50}$ = 3 μM. In $M. tuberculosis$ HN878, the phenyl (8h) and benzyl (8g) analogs had the same activity, IC$_{50}$ = 1.6 μM. Overall then, the SPR results do not support the idea that MmpL3 is the major target for SQ109 (8a) or analog activity in the mycobacteria, meaning that other targets such as uncoupling activity, or indeed other mechanisms of action, are involved, consistent of course with the activity (for SQ109) seen against other bacteria, as well as against yeasts, fungi and protozoa.
Membrane–inhibitor interactions. Another target for SQ109 (8a) in both bacteria and protozoa involves effects on the proton motive force, and Ca$$^{2+}$$ homeostasis—both of which involve cell membranes. In previous work 34 we investigated the pH dependence of the activity of inhibitors, including SQ109 analogs, on M. smegmatis cell growth inhibition (IC$_{50}$ values) and found that there were correlations between the IC$_{50}$ values and uncoupling (ΔpH collapse), as well as with changes in the gel-to-liquid crystal phase transition temperature ($T_m$) in DSC experiments with lipid bilayer membranes, and with computed logD values. We showed 34 that cell growth inhibition activity increased from pH 5 to 7 to 9, and this correlated with increasing uncoupler activity, decreasing $T_m$ values, and increasing logD values. That is, increased hydrophobicity results in more membrane binding leading to more fluidity and uncoupling activity, as well as providing a reservoir of inhibitor for binding to membrane protein targets. Such effects could be important in the protozoa.

We therefore next used DSC to investigate the effects of SQ109 (8a) and analogs 8b-g, 12 on the gel-to-liquid crystal phase transition in 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayers (10 wt % inhibitor, pH 7.4) as well as with 1,2-distearoyl-sn-glycero-3-phosphoglycerol (DSPG) bilayers. The zwitterionic DMPC is a model for protozoal membranes which are primarily phosphatidylcholines and phosphatidylethanolamines, while the DSPG is an anionic species and is a model for the mycobacterial inner membrane lipids. DSC scans of each compound during heating (Figures 3a,b, Table 5) and cooling (Figure S3, S4, Table S1) were then compared with measurements on DMPC or DSPG alone. The DSC scans of the main transition for the DMPC systems (Figure 3a) showed that compared to DMPC without any inhibitor, SQ109 (8a), 12 decreased $T_m$ by 3-4 °C, while 8b (Me), 8c (Et), 8d (Pr), 8e (Bu), 8f (Hex), 8g (Bn) and 8h (Ph) decreased $T_m$ by ca. 5.6, 6.6, 6, 7.8, 9.7, 6.9 and 8.8 °C, respectively (Table 5). The DSC scans for DSPG systems in Figure 3b showed that compared to DSPG alone SQ109 (8a), 8b (Me), 12 and 8d (Pr) decreased the $T_m$ by 3-4 °C, while 8e (Bu), 8f (Hex), 8g (Bn) and 8h (Ph) decreased $T_m$ by ~5 °C (Table 5).
These results indicated that SQ109 (8a) and the analogs incorporate into the phospholipid bilayers and fluidize the membranes, generating a structure that melts at a lower temperature, $T_m$, compared to the pure membrane, and this effect was more pronounced as the size of the inhibitor increases. Compared to the pure DMPC bilayer, SQ109 (8a) or 8b (Me), 8c (Et), 8d (Pr), 8e (Bu), 8h (Ph), 12 broadened the main transition peak by $\Delta T_{1/2} = 0.7-2.4 \, ^\circ$C (Table 5), while compounds 12, 8f (Hex) and 8g (Bn) broadened by $\Delta T_{1/2} = 3.5, 4.4, 9.6 \, ^\circ$C, due most likely to the formation of inhomogeneous domains that melt over a broad temperature range. Similarly, compared to the pure DSPG bilayer, SQ109 (8a), 8b (Me), 8c (Et), 8d (Pr), 8f (Hex), 8g (Bn) and 12 broadened the main transition peak by $\Delta T_{1/2} = 0.2-1.6 \, ^\circ$C; while compounds 8e (Bu) and 8h (Ph) broadened the peak by 2.7 and 4.4 $^\circ$C,
respectively, again due most likely to the formation of inhomogeneous domains that melt over a broad temperature range. However, rather than simply a very large broadening of the gel-to-liquid crystal phase transition, as seen with the fluidizing effect of cholesterol, in all cases \( T_m \) shifted to lower temperatures. The DSC thermograms obtained on cooling, Figure S3, S4 and Table S1, showed a similar decrease in \( T_m \) as seen with DMPC on heating but a more complex hysteresis effect with DSPG.

What is perhaps surprising is that, in general, the effects on \( T_m \) seen with DPMC were clearly larger than those seen with DSPG. What might the reasons for this be? Interestingly, similar effects have been reported for the binding of another cationic antibiotic, the anthracycline pirarubicin binding to DSPG and to distearoylphosphatidylcholine (DSPC), in which the acyl chain lengths are the same (C\(_{18}\)). In that work it was proposed (based on the results of DSC, Fourier transform infra-red spectroscopy and quantum chemical calculations) that the ammonium group of the antibiotic bound more tightly to the PO\(_2^-\) group in DSPG than to the PO\(_2^-\) group in DSPC, due to charge repulsion with the choline Me\(_3\)N\(^+\) group in DSPC, and that this resulted in decreased drug incorporation into the lipid bilayer. These observations led us to the following model for SQ109-DMPC/DSPG interactions. First, incorporation of the SQ109 geranyl (C\(_{10}\)) chain into the lipid bilayer was expected to decrease \( T_m \) in the same way that incorporation of a farnesyl (C\(_{15}\)) group in farnesol decreased and broadened the DMPC main transition, due to disrupted packing of the DMPC alkyl chains. A similar effect would be predicted for both DMPC as well as DSPG, but the effect would be larger with the shorter chain species, DMPC, which has a similar alkyl chain length to that of the geranyl chain in SQ109. The enthalpy of the phase transition was also of course much larger with the longer chain, DSPG, species, Table 5. However, there is a second interaction that may also be of importance, the electrostatic interaction between the PO\(_2^-\) group in the phospholipid and the protonated ethylenediamine group of SQ109 (8a) (or analog). Binding of cationic species (e.g. Ca\(^{2+}\), UO\(_2^{2+}\)) to anionic lipids increases \( T_m \) and it is possible that SQ109 (8a) may help “cross-link” the anionic DSPG, thereby off-setting to some extent the fluidizing effect of the geranyl group in SQ109 (8a).
**Table 5.** DSC results (heating) for the DMPC:SQ109 analog systems in DMPC hydrated with PBS (phosphate buffered saline, pH=7.4) and calculated logD_{7.4} values at the same pH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T_m (°C)</th>
<th>ΔT_{1/2} °C</th>
<th>ΔH (kJ mol^{-1})</th>
<th>Sample</th>
<th>T_m (°C)</th>
<th>ΔT_{1/2} °C</th>
<th>ΔH (kJ mol^{-1})</th>
<th>logD_{7.4}</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC</td>
<td>23.5</td>
<td>0.59</td>
<td>30.3</td>
<td>DSPG</td>
<td>53.6</td>
<td>0.9</td>
<td>48.1</td>
<td></td>
</tr>
<tr>
<td>DMPC:8a (H)</td>
<td>20.6</td>
<td>2.6</td>
<td>23.3</td>
<td>DSPG:8a (H)</td>
<td>50.4</td>
<td>2.1</td>
<td>54.0</td>
<td>1.39</td>
</tr>
<tr>
<td>DMPC:8b (Me)</td>
<td>17.9</td>
<td>2.2</td>
<td>25.6</td>
<td>DSPG:8b (Me)</td>
<td>50.5</td>
<td>2.0</td>
<td>53.2</td>
<td>1.40</td>
</tr>
<tr>
<td>DMPC:12</td>
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<td>4.1</td>
<td>17.8</td>
<td>DSPG:12</td>
<td>49.8</td>
<td>2.4</td>
<td>55.8</td>
<td>2.36</td>
</tr>
<tr>
<td>DMPC:8d (Pr)</td>
<td>17.5</td>
<td>2.2</td>
<td>22.6</td>
<td>DSPG:8d (Pr)</td>
<td>50.0</td>
<td>1.1</td>
<td>48.1</td>
<td>2.14</td>
</tr>
<tr>
<td>DMPC:8c (Et)</td>
<td>16.9</td>
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<td>DSPG:8c (Et)</td>
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<td>2.5</td>
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<td>24.4</td>
<td>DSPG:8e (Bu)</td>
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<td>DMPC:8f (Hex)</td>
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<td>21.6</td>
<td>DSPG:8g (Bn)</td>
<td>48.0</td>
<td>1.7</td>
<td>55.5</td>
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<td>DMPC:8h (Ph)</td>
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<td>48.6</td>
<td>5.3</td>
<td>55.4</td>
<td>3.52</td>
</tr>
</tbody>
</table>

m: main transition; T_m (°C), temperature at which heat capacity (ΔC_P) at constant pressure is a maximum; ΔT_{1/2} (°C), half width at half peak height of the main transition; ΔH (kJ mol^{-1}), main transition enthalpy normalized per g of the bilayer. Results were the same after two repeats.

What is also of particular interest about the DSC results shown in Figure 3a,b is that there are good correlations between the change in T_m that correlates with the computed logD_{7.4} of the inhibitor, Figures 3c and 3d, with a Pearson r-coefficient = 0.776, p = 0.014 for DMPC and a Pearson r-coefficient = 0.727, p = 0.026 for DSPG. For the cooling curves, there was again a correlation between ΔT_m and logD with DMPC (r = 0.75, p = 0.021) but there were more complex effects with DSPG (Figure S4). The stronger binding of the more hydrophobic analogs to lipid membranes would be expected to result in more cell activity, either by increasing membrane fluidity/uncoupling activity or by providing higher membrane concentrations for a membrane protein target. However, the more hydrophobic analogs were not found to be more active against *M. tuberculosis* or *M. smegmatis*, but we did find increased activity (a 4-8 fold decrease in MIC) in *M. abscessus*, as well as large increases in activity against *B. subtilis* and *E. coli*, Table 1, and with *P. falciparum* (with the phenyl and benzyl analogs). The DSC results thus suggest that the larger analogs may simply not reach their target(s) in *M. tuberculosis* and *M. smegmatis*, e.g. due to unfavorable steric interactions with the mycolyl-arabinogalactan-peptidoglycan cell wall.
Microsomal stability and solubility. Finally, we investigated the microsomal stability of analog 8b (Me) and analog 8h (Ph) as well as of SQ109 (8a) in human, mice and rat liver microsomes. We found that, as expected 40, 41 SQ109 (8a) was rapidly metabolized (Table S2). The methyl analog 8b was ~2-4x more stable (% remanent at 60 minutes) than was SQ109 (8a). The phenyl analog 8h had the same % remanent as SQ109 (8a) (at 60 minutes) in mice microsomes, though was more rapidly metabolized in human and rat liver microsomes. The intrinsic clearance rates (CLint) in mice microsomes were essentially the same as found with SQ109 for 8b and 8h but were about twice as large as found with SQ109 (8a) in the human and rat liver microsome assays (Table S2). While all of these rates are high, it is of course well known that SQ109 (8a) is, nevertheless, effective against *M. tuberculosis* in humans where it accumulates in the lungs, and in addition, it has efficacy against both trypanosomatid as well as apicomplexan parasites, in mice models of infection, 42 where the lungs are not targeted. Clearly, it will be of interest to determine how e.g. 8h is metabolized, and whether any metabolites have activity, and further work on this topic as well as on the synthesis of 8h analogs, is of interest. To determine the susceptibility of the phenyl group in 8h to oxygenation we used 3 computer programs: the GloryFame2 program, 43, 44 the Xenosite program 45 and the SmartCyp program. 46 Results are shown in Figure S5. Based on these results it is apparent that in general the major sites targeted by P450 cytochromes are—as with SQ109 (8a) itself—at the ends of the molecule (the terminal methyl groups and the adamantyl group) and at C10, but the phenyl group is also a potential target and may form a phenol (C19, Figure S5a, GloryFame2 result; Figure S5c, SmartCyp result) or a quinone (Figure S5b) moiety.

CONCLUSIONS

In this work we report the synthesis of analogs of the anti-tubercular drug candidate SQ109 (8a), an ethylenediamine-based inhibitor of MmpL3 currently undergoing clinical trials that also has activity against a broad range of bacteria, protozoa and even some yeasts/fungi. We synthesized a series of 19 SQ109 (8a) analogs containing adamantane C-2 alkyl, aryl or heteroaryl adamantyl groups (Me, Et, Pr, Bu, Ph, Bn, Hex, 4-phenylthiazol-2-yl, with ethylenediamine or aminoamide linkers between the adamantyl and geranyl groups, in addition to an adamantyl C-1 dimethylmethylene analog. We then tested SQ109 (8a) and the analogs against five bacteria: *M. smegmatis*, *M. tuberculosis* (3 strains), *M. abscessus* (two strains), *B. subtilis* and *E. coli*, as well as against five protozoa, *T. brucei*, *T. cruzi* (epimastigotes and amastigotes), *L. donovani*, *L. mexicana* and *P. falciparum* (asexual blood stages). There was good activity of ethylenediamine analogs containing small substituents against the mycobacteria, though activity was generally less than with the SQ109
parent molecule. The interesting exception was in *M. abscessus* where we found two SQ109 analogs, the butyl (8e) and benzyl (8g) species, in which activity was ~4-8 fold higher than found with SQ109 (8a). Moreover, activity was the same against a highly drug-resistant *M. abscessus* strain, harboring a A309P mutation located in the transmembrane domain in MmpL3, indicating that MmpL3 is not a major target in this species. We then used SPR to investigate the binding of SQ109, nine ethylenediamine analogs and four amide analogs to a mycobacterial MmpL3 protein target finding that tighter MmpL3 binding correlated with increasing ligand hydrophobicity with $r=0.73$, $p<0.003$ for the $\log K_D/\log D_{7.4}$ correlation. However, there were no significant correlations between cell growth inhibition and either $\log D$ or $\log K_D$. The low activity of the more potent MmpL3 binders against MmpL3-containing bacteria suggests that MmpL3 inhibitors with large C-2 substituents may not be able to penetrate the cell wall. Using DSC, we found that these inhibitors caused larger decreases in $T_m$ than found with SQ109 (8a), indicating efficient accumulation in lipid membranes and there were clear correlations between $\Delta T_m$ and $\log D_{7.4}$ for both DMPC ($r = 0.776$, $p = 0.014$) as well as for DSPG ($r = 0.727$, $p = 0.026$). Taken together, the SPR and DSC results are consistent with the idea that the larger, more potent MmpL3 inhibitors are less effective than is SQ109 (8a) in penetrating the arabinogalactan-peptidoglycan cell wall in mycobacteria. In contrast, in *B. subtilis* and *E. coli*, several analogs were more potent than was SQ109 (8a). In these organisms, MmpL3 is absent as is the mycolyl-arabinogalactan-peptidoglycan cell wall, although the mechanism of action of SQ109 (8a) itself in these bacteria remains to reported. On the other hand, in the trypanosomatid parasites, SQ109 (8a) is reported to act as a protonophore uncoupler that also affects $Ca^{2+}$ homeostasis. In these organisms there is again no cell wall, and many of the analogs are active against the parasites. With the malaria parasite *P. falciparum*, there is likewise no cell wall and no MmpL3 and SQ109 and analog activity could similarly be dependent on their protonophore properties. We found that several analogs had 4-10x increased activity over SQ109 as well as low toxicity against the HepG2 human cell line making them of interest as a new anti-malarial drug hits, warranting further development of these more-bulky analogs, as antimalarial drug leads.

**EXPERIMENTAL SECTION**

**General.** All solvents and chemicals were used as purchased without further purification except for Me$_3$SiCl which was distilled just before use. Dry ether and dry dichloromethane were prepared by leaving the solvents over CaH$_2$ for 24 h before use. Anhydrous THF was purchased in sealed bottles from Acros Organics. The progress of all reactions was monitored on Merck precoated silica gel plates (with fluorescence indicator UV254) using diethyl ether/n-hexane, n-hexane/ethyl acetate or
chloroform/methanol as eluents. Column chromatography was performed with Acros Organics silica gel 60A 40-60 μm with the solvent mixtures described in the experiment section. Compounds’ spots were visualized based on their absorbance at 254 nm. The structures of 8a-i, 12 were identified using 1H, 13C and LC-MS. High-resolution mass spectrometry (HRMS) was carried using a UHR-TOF maXis 4G instrument (Bruker Daltonics, Bremen, Germany). 1H NMR spectra were recorded in CDCl3 solutions for the amines and CD3OD solutions for the fumarate salts of amines on Bruker NMR spectrometers, the DRX 200 or DRX 400 or DRX 600 at 200 MHz or 400 or 600 MHz, respectively; 13C NMR spectra were recorded at 50 MHz, 100 or 150 MHz, respectively. Carbon multiplicities were assigned using the DEPT experiment. 2D NMR HMQC and COSY spectra were used for the elucidation of the structures of intermediates and final products and NMR peaks assignments. All compounds were confirmed to have greater than 95% purity via HPLC-MS analysis. We described an improved preparation of geranylamine (2) and the preparation of 6a, 7a, 13, 14a, 8a was also reported in ref; the preparation of geranylamine (2) and the raw materials for amines 5b-h, 12 were the corresponding adamantanol obtained from the reaction of 2-amantanone with alkyl lithium reagents. The tertiary alcohols were then reacted with a mixture of sodium azide and trifluoroacetic acid in dichloromethane and the produced tertiary alkyl azides were reduced with LiAlH4 in ether at rt to afford the amines 5b-h, 12. The 2-adamantylamine 5a was prepared from reduction of 2-adamantanone oxime with LiAlH4 in THF. The preparation of intermediates 2, 5i, 6a-i, 10, 13 and of 7a, 14a and SQ109 (8a) published in ref can be found in the Supporting Information.

2-((3,7-Dimethylocta-2,6-dien-1-yl)amino)-N-(2-methyl-2-adamantanyl)acetamide, 7b

![Chemical structure of 7b](attachment:image.png)

Bromoacetamide 6b (660 mg, 2.31 mmol) in dry THF (13 mL) was added dropwise at 0 °C to a stirred mixture of geranylamine (2) (353 mg, 2.31 mmol) and triethylamine (233 mg, 2.31 mmol) in dry THF (20 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl3:MeOH (9:1), as eluents. Acetamide 7b was obtained as a pale yellow oil; yield 744 mg (90%); 1H-NMR (CDCl3, 400 MHz) δ (ppm) 1.51 (s, 3H, 2-adamantyl-CH3), 1.59 (s, 3H, 8-geranyl), 1.64 (s, 3H, 7-geranyl CH3),
1.67 (s, 3H, 3-geranyl CH$_3$), 1.80 (s, 2H, 5,7-adamantyl), 1.96-2.05 (m, 6H, 4,5-H, 8ax,10ax-adamantyl), 2.18 (d, $J$ = 12 Hz, 2H, 4ax,9ax-adamantyl) 3.17 (d, $J$ = 7.4 Hz, 2H, 1-geranyl) 3.28-3.30 (m, 2H, COCH$_2$NH), 5.07 (m, 1H, 6-geranyl), 5.23 (m, 1H, 2-geranyl); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ (ppm) 16.7 (3-geranyl CH$_3$), 18.1 (8-geranyl), 23.5 (2-adamantyl-CH$_3$), 26.0 (7-geranyl CH$_3$), 27.2 (5-geranyl), 27.2 (5-adamantyl), 27.8 (7-adamantyl), 33.6 (4,9-adamantyl), 35.7 (1,3-adamantyl), 38.9 (8,10-adamantyl), 39.9 (6-adamantyl), 40.1 (4-geranyl), 47.3 (1-geranyl), 58.5 (2-adamantyl), 59.7 (COCH$_2$NH), 123.1 (2-geranyl), 124.1 (6-geranyl), 132.2 (7-geranyl), 138.5 (3-geranyl), 168.9 (C=O); HRMS (ESI-TOF (+)) $m/z$ [M + H]$^+$ calculated for [C$_{23}$H$_{39}$N$_2$O]$^+$ 359.3057, found 359.3062.

$N$-(3,7-dimethylocta-2,6-dien-1-yl)-$N'$-(2-methyl-2-adamantanyl)ethane-1,2-diamine, 8b

Acetamide 7b (790 mg, 2.21 mmol) in dry dichloromethane (10 mL) was stirred at 0-5 °C for 15 min under argon atmosphere. Recently distilled trimethylsilyl chloride (288 μL, 2.65 mmol) was then added at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH$_4$ (117 mg, 3.09 mmol) in a small quantity of THF was added at -10-0°C and the stirring continued for 2.5 h at the same temperature. The mixture was then treated with NaOH 10%, the resulting inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude product was dissolved in dichloromethane and washed with brine. After the evaporation of the solvent, the crude product was purified through column chromatography using a. CHCl$_3$:MeOH (9:1), b. CHCl$_3$:MeOH:NH$_3$ (88:10:2), as system solvents to afford diamine 8b as a pale yellow oil; yield 190 mg (25%); $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm) 1.21 (s, 3H, 2-adamantyl-CH$_3$), 1.51 (d, $J$ = 12Hz, 2H, 4eq,9eq-adamantyl), 1.53 (s, 2H, 8eq,10eq-adamantyl), 1.59 (s, 3H, 8-geranyl), 1.63 (s, 3H, 7-geranyl CH$_3$), 1.67 (s, 3H, 3-geranyl CH$_3$), 1.80 (d, $J$ = 12Hz, 2H, 5,7-adamantyl), 1.98-2.12 (m, 4H, 4,5-geranyl), 2.64 (t, $J$ = 6Hz, 2H, NHCH$_2$CH$_2$NH-geranyl), 2.74 (t, $J$ = 6Hz, 2H, NHCH$_2$CH$_2$NH-geranyl), 3.23 (d, $J$ = 7.0 Hz, 2H, 1-geranyl), 5.09 (m, 1H, 6-geranyl), 5.25 (m, 1H, 2-geranyl); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ (ppm) 16.6 (3-geranyl CH$_3$), 18.0 (8-geranyl ), 23.2 (2-adamantyl CH$_3$), 26.0 (7-adamantytyl CH$_3$), 26.9 (5-geranyl), 27.5 (5-adamantyl), 28.4 (7-adamantyl), 32.9 (4,9-adamantyl), 34.5 (1,3-adamantyl), 36.3 (8,10-adamantyl), 39.3 (6-adamantyl), 40.0 (4-geranyl), 40.2 (1-geranyl), 47.4 (NHCH$_2$CH$_2$NH-
geranyl), 50.1 (NHCH₂CH₂NH-geranyl), 56.3 (2-adamantyl), 123.3 (2-geranyl), 124.5 (6-geranyl), 131.8 (7-geranyl), 138.0 (3-geranyl); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₃H₄₁N₂]⁺ 345.3264, found 345.3269; Purity of the product determined by HPLC-MS: 96.4%.

\[N-(3,7\text{-dimethyl} oc\text{-2,6-} \text{dien-1-yl})-2-((2\text{-methyl-}2\text{-adamantanyl})\text{amino)acetamide, 14b}\]

Bromoacetamide 13 (664 mg, 2.42 mmol) in dry THF (14 mL) was added dropwise at 0 °C to a stirred solution of 2-methyl-2-adamantanamine (5b) (400 mg, 2.42 mmol) and triethylamine (244 mg, 2.42 mmol) in dry THF (24 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 14b was obtained as a yellow oil; yield 520 mg (60%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 1.14 (s, 3H, 2-adamantyl CH₃), 1.55 (d, J = 12Hz, 2H, 4eq,9eq-adamantyl), 1.59 (s, 3H, 8-geranyl), 1.67 (s, 6H, 3,7-geranyl CH₃), 1.91-1.94 (m, 2H, 4ax,9ax-adamantyl), 1.99-2.08 (m, 4H, 4,5-geranyl), 3.20 (s, 2H, NHCH₂CO), 3.86 (t, J = 6 Hz, 2H, 1-geranyl), 5.08 (m, 1H, 6-geranyl), 5.19 (m, 1H, 2-geranyl). Hydrochloride salt; ¹³C-NMR (CD₃OD, 100MHz) δ(ppm) 17.2 (3-geranyl CH₃), 18.6 (8-geranyl), 21.4 (2-adamanyl CH₃), 26.7 (7-geranyl CH₃), 28.3 (5-geranyl), 28.4 (5-adamantyl), 29.1 (7-adamantyl), 33.4 (4,9-adamantyl), 35.4 (8,10-adamantyl), 36.1 (1,3-adamantyl), 39.6 (6-adamantyl), 39.9 (4-geranyl), 41.4 (1-C), 43.3 (NHCH₂CO), 67.3 (2-adamantyl), 121.3 (2-geranyl), 125.8 (6-geranyl), 133.5 (7-geranyl), 142.1 (3-geranyl), 167.1 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₃H₄₉N₂O]⁺ 359.3057, found 359.3057; Purity of the product determined by HPLC-MS: 98.0%.

\[N-(2\text{-adamantan-1-yl)propan-2-yl})-2-((3,7\text{-dimethyl} oc\text{-2,6-} \text{dien-1-yl})\text{amino)acetamide, 11}\]


Bromoacetamide 10 (680 mg, 2.16 mmol) in dry THF (15 mL) was added dropwise at 0 °C to a stirred solution of geranylamine (2) (330 mg, 2.16 mmol) and triethylamine (218 mg, 2.16 mmol) in dry THF (20 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 11 was obtained as a yellow oil; yield 750 mg (90%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 1.34 (s, 6H, 1-adamantyl C(CH₃)₂), 1.59-1.71 (m, 21H, 2,4,6,8,9,10-adamantyl, 8-geranyl, 3,7-geranyl CH₃), 2.01-2.09 (m, 7H, 3,5,7-adamantyl, 4,5-geranyl), 3.15 (d, J = 7.4 Hz, 2H, 1-geranyl), 3.23-3.26 (m, 2H, COC₃H₆), 5.07 (m, 1H, 6-geranyl), 5.22 (m, 1H, 2-geranyl); ¹³C-NMR (CDCl₃, 100MHz) δ(ppm) 16.7 (3-geranyl CH₃), 18.0 (8-geranyl), 21.4 (1-adamantyl C(CH₃)₂), 21.9 (1-adamantyl C(CH₃)₂), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl), 28.9 (3,5,7-adamantyl C), 36.5 (4,6,10-adamantyl), 37.4 (2,8,9-adamantanyl), 39.5 (1-adamantanyl), 39.8 (4-geranyl), 40.0 (1-geranyl), 47.3 (COCH₂NH), 59.01 (1-adamantanyl C(CH₃)₂), 119.8 (2-geranyl), 124.2 (6-geranyl), 132.1 (7-geranyl), 140.4 (3-geranyl), 169.1 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₅H₄₃N₂O]⁺ 387.337, found 387.337; Purity of the product determined by HPLC-MS: 99.2%.

\[N-\{(3,7\text{-dimethylocta-2,6-dien-1-yl})-N'-(2\{-adamantan-1-yl\}propan-2-yl)ethane-1,2\text{-diamine, 12}\]

Acetamide 11 (280 mg, 0.72 mmol) in dry dichloromethane (4 mL) was stirred at 0-5 °C for 15 min under argon atmosphere of Ar. Recently distilled trimethylsilyl chloride (94 μL, 0.87 mmol) was then added at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH₄ (38.3 mg, 1.01 mmol) in a small quantity of THF was added at -10-0°C and the stirring continued for 2.5 h at the same temperature. The mixture was treated with NaOH 10%, the resulting inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude product was dissolved in dichloromethane and washed with brine. After the evaporation of the solvent, the crude product was purified through column chromatography using a. CHCl₃:MeOH (9:1), b. CHCl₃:MeOH:NH₃ (88:10:2), as system solvents, to afford diamine 12 as a yellow oil; yield 70mg (26%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.96 (s, 6H, 1-adamantyl C(CH₃)₂), 1.60-1.67 (m, 21H,
2,4,6,8,9,10-adamantane H, 8-geranyl, 3,7-geranyl CH₃), 2.01-2.09 (m, 7H, 3,5,7-adamantyl, 4,5-geranyl), 2.70 (s, 4H, NHCH₂CH₂NH), 3.23 (d, J = 7.0 Hz, 2H, 1-geranyl), 5.09 (m, 1H, 6-geranyl), 5.26 (m, 1H, 2-geranyl); ¹³C NMR (CDCl₃, 100MHz) δ(ppm) 16.6 (3-geranyl CH₃), 18.0 (8-geranyl), 20.7 (1-adamantyl C(CH₃)₂), 26.0 (7-geranyl CH₃), 26.9 (5-geranyl), 29.2 (3,7,5-adamantyl), 36.5 (4,6,10-adamantyl), 37.6 (2,8,9-adamantyl), 39.2 (1-adamantyl), 40.0 (4-geranyl), 41.9 (1-geranyl), 47.2 (NHCH₂CH₂NH-geranyl), 50.2 (NHCH₂CH₂NH-geranyl), 57.2 (1-adamantyl C(CH₃)₂), 123.0 (2-geranyl), 124.5 (6-geranyl), 131.9 (7-geranyl), 138.3 (3-geranyl); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₅H₄₅N₂]+ 373.3577, found 373.357; Purity of the product determined by HPLC-MS: 96.6%.

2-((2-(Adamantan-1-yl)propan-2-yl)amino)-N-(3,7-dimethylocta-2,6-dien-1-yl)acetamide, 15

![Structure of 15](image)

Bromoacetamide 13 (200 mg, 0.73 mmol) in dry THF (6 mL) was added dropwise at 0 °C to a stirred solution of 2-(1-adamantanyl)propan-2-amine (9) (141 mg, 0.73 mmol) and triethylamine (74 mg, 0.73 mmol) in dry THF (8 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 15 was obtained as a yellow oil; yield 125 mg (44%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.92 (s, 6H, 1-adamantyl C(CH₃)₂), 1.59-1.73 (m, 21H, 2,4,6,8,9,10-adamantyl, 8-geranyl, 3,7-geranyl CH₃), 2.01-2.10 (m, 7H, 3,5,7-adamantyl, 4,5-geranyl), 3.28 (s, 2H, NHCH₂CO), 3.85 (t, J = 6.2 Hz, 2H, 1-geranyl), 5.08 (m, 1H, 6-geranyl), 5.21 (m, 1H, 2-geranyl); ¹³C-NMR (CDCl₃, 100MHz) δ(ppm) 16.7 (3-geranyl CH₃), 18.0 (8-geranyl), 20.3 (1-adamantyl C(CH₃)₂), 26.0 (7-geranyl CH₃), 26.9 (5-geranyl), 29.0 (3,5,7-adamantyl), 36.5 (4,6,10-adamantyl), 37.4 (2,8,9-adamantyl), 37.9 (1-adamantyl), 39.0 (4-geranyl), 39.8 (1-geranyl), 46.1 (NHCH₂CO), 120.3 (2-geranyl), 124.2 (6-geranyl), 132.0 (7-geranyl), 140.2 (3-geranyl), 169.2 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₅H₄₃N₂O]+ 387.337, found 387.3369; Purity of the product determined by HPLC-MS: 95.8%.

2-((3,7-Dimethylocta-2,6-dien-1-yl)amino)-N-(2-ethyladamantan-2-yl)acetamide, 7c
Bromoacetamide 6c (680 mg, 2.26 mmol) in dry THF (15 mL) was added dropwise at 0 °C to a stirred solution of geranylamine (2) (346 mg, 2.26 mmol) and triethylamine (228 mg, 2.26 mmol) in dry THF (20 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 7c was obtained as a pale yellow oil; yield 710 mg (84%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.75 (t, J = 7Hz, 3H, 2-adamantyl CH₂), 1.60 (s, 3H, 8-geranyl), 1.63 (s, 3H, 7-geranyl CH₃), 1.68 (s, 3H, 3-geranyl CH₃), 1.60-1.70 (m, 6H, 1,3,4eq,8eq,9eq,10eq-adamantyl), 1.81 (s, 2H, 6-adamantyl), 1.93-2.11 (m, 10H, 5,7,8ax,10ax-adamantyl, 2-adamantyl CH₂CH₃, 4,5-geranyl), 2.25 (s, 2H, 4ax,9ax-adamantyl), 3.19 (s, 2H, COCH₂NH), 3.23 (d, J = 7.4 Hz, 2H, 1-geranyl), 5.07 (t, J = 7.4 Hz, 1H, 2-geranyl); ¹³C-NMR (CDCl₃, 100MHz) δ (ppm) 7.43 (2-adamantyl CH₂CH₃), 16.6 (3-geranyl CH₃), 18.0 (8-geranyl C), 25.0 (2-adamantyl CH₂CH₃), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl C), 27.6 (5,7-adamantyl), 33.2 (4,9-adamantyl), 33.1 (1,3-adamantyl), 33.7 (8,10-adamantyl), 39.0 (6-adamantyl), 40.0 (4-geranyl), 47.7 (1-geranyl), 52.7 (COCH₂NH), 60.4 (2-adamantyl), 122.2 (2-geranyl), 123.6 (6-geranyl), 132.1 (7-geranyl), 139.3 (3-geranyl), 170.3 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]^+ calculated for [C₂₀H₄₁N₂O]^+ 373.3213, found 373.3213; Purity of the product determined by HPLC-MS: 99.2%.

N-(3,7-dimethylocta-2,6-dien-1-yl)-N’-(2-ethyladamantan-2-yl)ethane-1,2-diamine, 8c

Acetamide 7c (420 mg, 1.13 mmol) in dry dichloromethane (5 mL) was stirred at 0-5 °C for 15 min under argon atmosphere. Recently distilled trimethylsilyl chloride (147 µL, 1.35 mmol) was then added at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH₄ (60 mg, 1.58 mmol) in a small quantity of THF was added at -10-0°C and the stirring continued for 2.5 h at the same temperature. The mixture was then treated with NaOH 10%, the resulting
inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude product was dissolved in dichloromethane and washed with brine. After evaporation of the solvent, the crude product was purified through column chromatography using a gradient of CHCl₃:MeOH (20:1, 18:1), as system solvents, to afford diamine 8c as a pale yellow oil; yield 150 mg (37%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.75 (t, J = 7 Hz, 3H, 2-adamantyl CH₂CH₃), 1.48 (d, J = 12 Hz, 2H, 4eq,9eq-adamantyl), 1.59 (s, 3H, 8-geranyl), 1.63 (s, 3H, 7-geranyl CH₃), 1.67 (s, 3H, 3-geranyl CH₃), 1.59-1.67 (m, 4H, 1,3,8eq,10eq-adamantyl), 1.80 (s, 2H, 6-adamantyl), 1.90 (d, J = 12 Hz, 2H, 5,7-adamantyl), 1.99-2.18 (m, 10H, 4ax,8ax,9ax,10ax-adamantyl, 2-adamantyl CH₂CH₃, 4,5-H), 2.54 (t, J = 6Hz, 2H, NHCH₂CH₂NH-geranyl), 2.74 (t, J = 6 Hz, 2H, NHCH₂CH₂NH-geranyl), 3.26 (d, J = 7.0 Hz, 2H, 1-geranyl), 5.08 (t, J = 7 Hz, 1H, 6-geranyl), 5.26 (t, J = 7 Hz, 1H, 2-geranyl); ¹³C NMR (CDCl₃, 100MHz) δ (ppm) 6.6 (2-adamantyl CH₂CH₂C₃H₃), 16.6 (3-geranyl CH₃), 18.1 (8-geranyl), 24.0 (2-adamantyl CH₂CH₃), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl), 28.1 (5,7-adamantyl), 32.9 (4,9-adamantyl), 33.9 (1,3,8,10-adamantyl), 39.4 (6-adamantyl), 40.0 (1,4-geranyl), 47.2 (NHCH₂CH₂NH-geranyl), 49.9 (NHCH₂CH₂NH-geranyl), 57.7 (2-adamantyl), 122.7 (2-geranyl), 124.4 (6-geranyl), 131.9 (7-geranyl), 138.5 (3-geranyl); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₄H₄₃N₂]⁺ 359.3421, found 359.342; Purity of the product determined by HPLC-MS: 95.9%.

2-((3,7-Dimethylocta-2,6-dien-1-yl)amino)-N-(2-propyladamantan-2-yl)acetamide, 7d

Bromoacetamide 6d (850 mg, 2.70 mmol) in dry THF (15 mL) was added dropwise at 0 °C to a stirred solution of geranylamine (2) (413 mg, 2.70 mmol) and triethylamine (273 mg, 2.70 mmol) in dry THF (25 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 7d was obtained as a pale yellow oil; yield 750 mg (72%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.89 (t, J = 7.4 Hz, 3H, 2-adamantyl CH₂CH₂CH₃), 1.12-1.25 (m, 2H, 2-adamantyl CH₂CH₂CH₃), 1.58-1.71 (m, 21H, 1,3,4eq,8eq,9eq,10eq-adamantyl, 8-geranyl, 3,7-geranyl CH₃, 2-adamantyl CH₂CH₂CH₃), 1.81 (s, 2H, 6-adamantyl), 1.96-2.05 (m, 8H, 5,7,8ax,10ax-adamantyl, 4,5-
geranyl), 2.24 (d, J = 12 Hz, 2H, 4ax,9ax-adamantyl) 3.19 (d, J = 7.4 Hz, 2H, 1-geranyl) 3.30-3.31 (m, 2H, COCH₂NH), 5.07 (m, 1H, 6-geranyl), 5.24 (m, 1H, 2-geranyl); ¹³C-NMR (CDCl₃, 100MHz) δ (ppm) 14.9 (2-adamantyl CH₂CH₂CH₃), 15.0 (2-adamantyl CH₂CH₂CH₃), 16.4 (3-geranyl CH₃), 18.0 (8-geranyl), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl), 27.6 (5,7-adamantyl), 33.3 (4,9-adamantyl), 33.5 (8,10-adamantyl), 33.7 (1,3-adamantyl), 33.9 (2-adamantyl CH₂CH₂CH₃), 35.4 (6-adamantyl), 39.0 (4-geranyl), 40.0 (1-geranyl), 47.5 (COCH₂NH), 52.0 (2-adamantyl), 119.7 (2-geranyl), 124.2 (6-geranyl), 132.1 (7-geranyl), 140.1 (3-geranyl), 168.7 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₅H₄₃N₂O]⁺ 387.337, found 387.337.

\[N-(3,7\text{-dimethyllocta-2,6-dien-1-yl})\text{-N'-(2-propyladamantan-2-yl)}\text{ethane-1,2-diamine, 8d}\]

Acetamide 7d (1.22 g, 3.16 mmol) in dry dichloromethane (14 mL) was stirred at 0-5 °C for 15 min under argon atmosphere. Recently distilled trimethylsilyl chloride (412 μL, 3.79 mmol) was then added at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH₄ (168 mg, 4.42 mmol) in a small quantity of THF was added at -10-0°C and the stirring continued for 2.5 h at the same temperature. The mixture was then treated with NaOH 10%, the resulting inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude product was dissolved in dichloromethane and washed with brine. After the evaporation of the solvent, the mixture was purified through column chromatography using a. CHCl₃:MeOH (9:1), b. CHCl₃:MeOH:NH₃ (88:10:2), as system solvents to afford diamine 8d as a pale yellow oil; yield 390 mg (33%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.90 (t, J = 7.4 Hz, 3H, 2-adamantyl CH₂CH₂CH₃), 1.15-1.25 (m, 2H, 2-adamantyl CH₂CH₂CH₃), 1.45 (d, J = 12 Hz, 2H, 4eq,9eq-adamantyl), 1.55-1.72 (m, 17H, 1,3,6,8eq,10eq-adamantyl, 8-geranyl, 3,7-geranyl CH₃, 2-adamantyl CH₂CH₂CH₃), 1.79 (d, J = 12 Hz, 2H, 5,7-adamantyl), 1.92 (d, J = 11.4 Hz, 2H, 8ax,10ax-adamantyl), 1.90-2.02 (m, 2H, 5-geranyl), 2.06-2.11 (m, 2H, 4-geranyl), 2.15 (d, J = 12.5 Hz, 4ax,9ax-adamantyl), 2.52 (t, J = 6Hz, 2H, NHCH₂CH₂NH-geranyl), 2.69 (t, J = 6 Hz, 2H, NHCH₂CH₂NH-geranyl), 3.23 (d, J = 7.0 Hz, 2H, 1-geranyl), 5.08 (t, J = 7 Hz, 1H, 6-geranyl), 5.25 (t, J = 7 Hz, 1H, 2-geranyl); ¹³C NMR (CDCl₃, 100MHz) δ (ppm) 15.2 (2-adamantyl CH₂CH₂CH₃), 15.3 (2-adamantyl CH₂CH₂CH₃), 16.6 (3-geranyl CH₃), 18.0 (8-geranyl), 26.0
(7-geranyl CH₃), 26.9 (5-geranyl C), 28.1 (5-adamantyl), 28.3 (7-adamantyl), 33.0 (4,9-adamantyl), 34.0 (8,10-adamantyl), 34.4 (1,3-adamantyl), 34.6 (2-adamantyl CH₂CH₂CH₃), 39.5 (6-adamantyl), 39.7 (4-geranyl), 40.0 (1-geranyl), 47.5 (NHCH₂CH₂NH-geranyl), 50.5 (NHCH₂CH₂NH-geranyl), 57.5 (2-adamantyl), 123.3 (2-(geranyl), 124.5 (6-geranyl), 131.8 (7-(geranyl), 137.9 (3-geranyl); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₅H₄₅N₂]⁺ 373.3577, found 373.3575; Purity of the product determined by HPLC-MS: 100.0%.

N-(3,7-dimethylocta-2,6-dien-1-yl)-2-((2-propyladamantan-2-yl)amino)acetamide, 14d

Acetamide 13 (184 mg, 0.67 mmol) in dry THF (6 mL) was added dropwise at 0 °C to a stirred solution of 2-propyl-2-adamantanamine (5d) (130 mg, 0.67 mmol) and triethylamine (68 mg, 0.67 mmol) in dry THF (8 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 14d was obtained as a pale yellow oil; yield 90 mg (35%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.90 (t, J = 7.4 Hz, 3H, 2-adamantyl CH₂CH₂CH₃), 1.14-1.20 (m, 2H, 2-adamantyl CH₂CH₂CH₃), 1.51-1.68 (m, 21H, 1,3,4,6,8eq,9,10eq-adamantyl, 8-geranyl, 3,7-geranyl CH₃, 2-adamantyl CH₂CH₂CH₃), 1.83 (s, 2H, 5,7-adamantyl), 1.90-1.93 (m, 2H, 4ax,9ax-adamantyl), 2.01-2.09 (m, 4H, 4,5-geranyl), 3.12 (s, 2H, NHCH₂CO), 3.87 (t, J = 6 Hz, 2H, 1-geranyl), 5.08 (t, J = 7 Hz, 1H, 6-geranyl), 5.20 (t, J = 7 Hz, 1H, 2-geranyl); ¹³C-NMR (CDCl₃, 100MHz) δ (ppm) 15.0 (2-adamantyl CH₂CH₂CH₃), 15.5 (2-adamantyl CH₂CH₂CH₃), 16.6 (3-geranyl CH₃), 18.0 (8-geranyl), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl), 28.0 (5,7-geranyl), 33.2 (4,9-adamantyl), 33.8 (8,10-adamantyl), 34.4 (1,3-adamantyl), 35.2 (2-adamantyl CH₂CH₂CH₃), 37.2 (6-adamantyl), 39.3 (4-geranyl), 39.8 (1-geranyl), 44.2 (NHCH₂CO), 120.4 (2-geranyl), 124.2 (6-geranyl), 132.1 (7-geranyl), 140.1 (3-geranyl), 168.8 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₅H₄₅N₂O]⁺ 387.337, found 387.3369; Purity of the product determined by HPLC-MS: 98.3%.

2-((3,7-Dimethylocta-2,6-dien-1-yl)amino)-N-(2-butyladamantan-2-yl)acetamide, 7e
Bromoacetamide 6e (190 mg, 0.58 mmol) in dry THF (4 mL) was added dropwise at 0 °C to a stirred solution of geranylamine (2) (89 mg, 0.58 mmol) and triethylamine (58 mg, 0.58 mmol) in dry THF (5 mL). The stirring continued for 72 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 7e was obtained as a pale yellow oil; yield 160 mg (69%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.73-0.77 (m, 3H, 2-adamantyl (CH₂)₃CH₃), 1.08-1.34 (m, 4H, 2-adamantyl CH₂(CH₂)₂CH₃), 1.60 (s, 3H, 8-geranyl), 1.63 (s, 3H, 7-geranyl CH₃), 1.68 (s, 3H, 3-geranyl CH₃), 1.59-1.71 (m, 6H, 1,3,4eq,8eq,9eq,10eq-adamantyl), 1.82 (s, 2H, 6-adamantyl), 1.89-2.08 (m, 10H, 5,7,8ax,10ax-adamantyl, 2-adamantyl CH₂(CH₂)₂CH₃, 4,5-geranyl), 2.24 (d, J = 12.5 Hz, 2H, 4ax,9ax-adamantyl), 3.20 (d, J = 7.4 Hz, 2H, 1-geranyl), 3.30 (s, 2H, COCH₂NH), 5.07 (t, J = 7.4 Hz, 1H, 6-geranyl), 5.24 (t, J = 7.4 Hz, 1H, 2-geranyl). Fumarate salt; ¹³C-NMR (MeOD, 100MHz, ) δ (ppm) 14.5 (2-adamantyl (CH₂)₃CH₃), 15.6 (3-geranyl CH₃), 16.8 (8-geranyl), 18.0 (7-geranyl CH₃), 23.5 (4,9-adamantyl), 25.4 (5-geranyl), 26.0 (2-adamantyl (CH₂)₂CH₂CH₃), 26.8 (2-adamantyl CH₂CH₂CH₂CH₃), 27.6 (5,7-adamantyl), 32.7 (2-adamantyl CH₂(CH₂)₂CH₃), 33.3 (8,10-adamantyl), 33.7 (1,3-adamantyl), 39.0 (6-adamantyl), 40.0 (4-geranyl), 47.3 (1-geranyl), 60.8 (COCH₂NH), 66.2 (2-adamantyl), 119.8 (2-geranyl), 124.1 (6-geranyl), 132.2 (3,7-geranyl), 168.7 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₆H₄₅N₂O]⁺ 401.3526, found 401.3527; Purity of the product determined by HPLC-MS: 98.6%.

*N-(3,7-dimethylocta-2,6-dien-1-yl)-N’-(2-butyladamantan-2-yl)ethane-1,2-diamine, 8e*

Acetamide 7e (160 mg, 0.40 mmol) in dry dichloromethane (2 mL) was stirred at 0-5 °C for 15 min under argon atmosphere. Recently distilled trimethylsilyl chloride (52 μL, 0.48 mmol) was then added
at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH₄ (21 mg, 0.56 mmol) in a small quantity of THF was added at -10-0°C and the stirring continued for 2.5 h at the same temperature. The mixture was then treated with NaOH 10%, the resulting inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude product was dissolved in dichloromethane and washed with brine. After the evaporation of the solvent, the crude product was purified through column chromatography using ether:n-hexane (1:1) and a gradient of CHCl₃:MeOH (25:1, 20:1), as system solvents to afford diamine 8e as a pale yellow oil; yield 20 mg (13%). Fumarate salt; ¹H-NMR (MeOD, 400 MHz) δ (ppm) 0.91 (t, J = 7Hz, 3H, 2-adamantyl (CH₂)₃CH₃), 1.12-1.33 (m, 4H, 2-adamantyl CH₂(CH₂)₂CH₃), 1.49 (d, J = 12Hz, 2H, 4eq,9eq-adamantyl), 1.59 (s, 3H, 8-geranyl), 1.64 (s, 3H, 7-geranyl CH₃), 1.67 (s, 3H, 3-geranyl CH₃), 1.59-1.69 (m, 8H, 1,3,6,8eq,10eq-adamantyl, 2-adamantyl-CH₂(CH₂)₂CH₃), 1.81 (s, 2H, 5,7-geranyl), 1.92 (d, J = 12Hz, 2H, 8ax,10ax-adamantyl), 1.99-2.11 (m, 4H, 4,5-geranyl), 2.16 (d, J =12Hz, 2H, 4ax,9ax-adamantyl), 2.59 (t, J = 6Hz, 2H, NHCH₂CH₂NH-geranyl), 2.79 (t, J = 6 Hz, 2H, NHCH₂CH₂NH-geranyl), 3.29 (d, J = 7.0 Hz, 2H, 1-geranyl), 5.08 (t, J = 7 Hz, 1H, 6-geranyl), 5.26 (t, J = 7 Hz, 1H, 2-geranyl); ¹³C NMR (MeOD, 100MHz) δ (ppm) 15.4 (2-adamantyl (CH₂)₃CH₃), 17.5 (3-geranyl CH₃), 18.7 (8-geranyl), 19.7 (7-geranyl CH₃), 23.4 (5-geranyl), 25.2 (4-adamantyl), 25.7 (9-adamantyl), 26.7 (2-adamantyl (CH₂)₂CH₂CH₃), 28.1 (2-adamantyl CH₂CH₂CH₂CH₃), 29.8 (5,7-adamantyl), 33.1 (2-adamantyl CH₂(CH₂)₂CH₃), 34.1 (8-adamantyl), 35.4 (10-adamantyl), 35.7 (1,3-adamantyl), 38.9 (6-adamantyl), 40.8 (4-geranyl), 41.6 (1-geranyl), 47.3 (NHCH₂CH₂NH-geranyl), 48.2 (NHCH₂CH₂NH-geranyl), 62.1 (2-adamantyl), 117.2 (2-geranyl), 125.6 (6-geranyl), 136.7 (3,7-geranyl); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₆H₄₇N₂]⁺ 387.3734, found 387.3737; Purity of the product determined by HPLC-MS: 98.2%.

2-((3,7-Dimethylocta-2,6-dien-1-yl)amino)-N-(2-hexyladamantan-2-yl)acetamide, 7f
Bromoacetamide 6f (70 mg, 0.20 mmol) in dry THF (1.5 mL) was added dropwise at 0 °C to a stirred solution of geranylamine (2) (31 mg, 0.20 mmol) and triethylamine (20 mg, 0.20 mmol) in dry THF (2 mL). The stirring continued for 72 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 7f was obtained as a yellow oil; yield 50 mg (60%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.88 (t, J = 7Hz, 3H, 2-adamantyl (CH₂)₅CH₃), 1.11-1.33 (m, 8H, 2-adamantyl CH₂(CH₂)₄CH₃), 1.51-1.68 (m, 19H, 1,3,4eq,6,8eq,9eq,10eq-adamantyl, 3-geranyl CH₃, 7-geranyl CH₃, 8-H, 2-adamantyl CH₂(CH₂)₄CH₃), 1.82-1.83 (m, 2H, 5,7-adamantyl), 1.91 (d, J = 8.5 Hz, 2H, 8ax,10ax-adamantyl), 2.01-2.10 (m, 6H, 4ax,9ax-adamantyl, 4,5-geranyl), 3.11 (s, 2H, COC₂H₂NH), 3.88 (t, J = 4 Hz, 2-geranyl), 5.08 (t, J = 7.4 Hz, 1H, 6-geranyl), 5.21 (t, J = 7.4 Hz, 1H, 2-geranyl); ¹³C-NMR (CDCl₃, 100MHz) δ (ppm) 15.6 (2-adamantyl (CH₂)₅CH₃), 16.6 (3-geranyl CH₃), 18.0 (8-geranyl), 22.2 (2-adamantyl (CH₂)₄CH₂CH₃), 23.0 (2-adamantyl (CH₂)₃CH₂CH₂CH₂CH₃), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl), 28.0 (5,7-adamantyl), 30.3 (2-adamantyl CH₂(CH₂)₂CH₂(CH₂)₂CH₃), 32.4 (2-adamantyl CH₂CH₂(CH₂)₂CH₃), 32.7 (2-adamantyl CH₂(CH₂)₃CH₃), 33.3 (4,9-adamantyl), 33.9 (8,10-adamantyl), 34.4 (1,3-adamantyl), 37.2 (6-adamantyl), 39.3 (4-geranyl), 39.8 (1-geranyl), 58.0 (COCH₂NH), 66.2 (2-adamantyl), 120.5 (2-geranyl), 124.2 (6-geranyl), 132.1 (7-geranyl), 140.1 (3-geranyl), 172.8 (C=O).

**N-(3,7-dimethyl-octa-2,6-dien-1-yl)-N’-(2-hexyladamantan-2-yl)ethane-1,2-diamine, 8f**

![Chemical structure of 8f](image)

Acetamide 7f (50 mg, 0.12 mmol) in dry dichloromethane (1 mL) was stirred at 0-5 °C for 15 min under argon atmosphere. Recently distilled trimethylsilyl chloride (15 μL, 0.14 mmol) was then added at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH₄ (7 mg, 0.17 mmol) in a small quantity of THF was added at -10-0 °C and the stirring continued for 2.5 h at the same temperature. The mixture was then treated with NaOH 10%, the resulting inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude was dissolved in dichloromethane and washed with brine. After the evaporation of the solvent, the crude product was purified through column chromatography using ether:n-hexane (1:1) and...
CHCl₃:MeOH (30:1), as system solvents to afford diamine 8f as a yellow oil; yield 20 mg (40%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 0.87 (t, J = 7Hz, 3H, 2-adamantyl (CH₂)₃CH₃), 1.18-1.27 (m, 8H, 2-adamantyl CH₂(CH₂)₄CH₃), 1.49 (d, J = 12Hz, 2H, 4eq,9eq-adamantyl), 1.59 (s, 3H, 8-geranyl), 1.64 (s, 3H, 7-geranyl CH₃), 1.66 (s, 3H, 3-geranyl CH₃), 1.59-1.69 (m, 8H, 1,3,6,8eq,10eq-adamantyl, 2-adamantyl CH₂(CH₂)₄CH₃), 1.81 (s, 2H, 5,7-adamantyl), 1.91 (d, J = 8.5 Hz, 2H, 8ax,10ax-adamantyl), 1.99-2.10 (m, 4H, 4,5-geranyl), 2.16 (d, J = 8.5 Hz, 2H, 4ax,9ax-adamantyl), 2.58 (t, J = 6Hz, 2H, NHCH₂CH₂NH-geranyl), 2.78 (t, J = 6 Hz, 2H, NHCH₂CH₂NH-geranyl), 3.29 (d, J = 7.0 Hz, 2H, 1-geranyl), 5.07 (t, J = 7 Hz, 1H, 6-geranyl), 5.25 (t, J = 7 Hz, 1H, 2-geranyl); ¹³C NMR (CDCl₃, 100MHz) δ (ppm) 14.4 (2-adamantyl (CH₂)₃CH₃), 16.7 (3-geranyl CH₃), 18.0 (8-geranyl), 22.0 (2-adamantyl (CH₂)₄CH₂CH₃), 23.1 (2-adamantyl (CH₂)₃CH₂CH₂CH₃), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl), 27.9 (5,7-adamantyl), 30.4 (2-adamantyl CH₂CH₂CH₂CH₂CH₂CH₃), 32.0 (2-adamantyl CH₂CH₂(CH₂)₃CH₃), 32.3 (2-adamantyl CH₂(CH₂)₄CH₃), 32.9 (4,9-adamantyl), 33.9 (8,10-adamantyl), 34.2 (1,3-adamantyl), 39.1 (6-adamantyl), 39.3 (4-geranyl), 40.0 (1-geranyl), 46.9 (NHCH₂CH₂NH-geranyl), 49.2 (NHCH₂CH₂NH-geranyl), 66.2 (2-adamantyl), 121.9 (2-geranyl), 124.3 (6-geranyl), 131.9 (7-geranyl), 139.2 (3-geranyl); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₈H₅₁N₂]⁺ 415.4047, found 415.404; Purity of the product determined by HPLC-MS: 100.0%.

2-([(3,7-Dimethylocta-2,6-dien-1-yl)amino]-N-(2-benzyladamantan-2-yl)acetamide, 7g

Bromoacetamide 6g (530 mg, 1.46 mmol) in dry THF (8 mL) was added dropwise at 0 ºC to a stirred solution of geranylamine (2) (223 mg, 1.46 mmol) and triethylamine (147 mg, 1.46 mmol) in dry THF (12.5 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 7g was obtained as a pale yellow oil; yield 560 mg (88%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 1.57 (s, 3H, 8-geranyl), 1.59 (s, 3H, 7-geranyl CH₃), 1.67 (s, H, 3-geranyl CH₃), 1.57-1.84 (m, 9H, 1,3,4eq,5,6eq,7,8eq,9eq,10eq-adamantyl), 1.90 (s, 1H, 6ax-adamantyl), 1.98-2.08 (m, 4H, 4,5-geranyl), 2.24-2.30 (m, 4H, 4ax,8ax,9ax,10ax-adamantyl), 3.14 (d,
J = 7.4 Hz, 2H, 1-geranyl), 3.25 (s, 2H, COCH₂NH), 3.38 (s, 2H, benzyl), 5.07 (t, J = 7.4 Hz, 1H, 6-geranyl), 5.26 (t, J = 7.4 Hz, 1H, 2-geranyl), 7.06-7.14 (m, 2H, phenyl), 7.16-7.19 (m, 1H, phenyl), 7.22-7.25 (m, 2H, phenyl); ¹³C-NMR (CDCl₃, 100MHz) δ (ppm) 16.8 (3-geranyl CH₃), 18.0 (8-geranyl), 26.0 (7-geranyl CH₃), 26.7 (5-geranyl), 27.3 (5-adamantyl), 27.8 (7-adamantyl), 33.5 (4,8,9,10-adamantyl), 33.6 (1,3-adamantyl), 38.1 (benzyl), 38.9 (6-adamantyl), 39.9 (4-geranyl), 47.1 (1-geranyl), 56.7 (COCH₂NH), 62.0 (2-adamantyl), 123.9 (6-geranyl), 126.5 (phenyl), 128.3 (phenyl), 130.6 (phenyl), 138.5 (quaternary-phenyl), 163.6 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₂₉H₄₃N₂O]⁺ 435.337, found 435.3371; Purity of the product determined by HPLC-MS: 97.8%.

N-(3,7-dimethylocta-2,6-dien-1-yl)-N’-(2-benzyladamantan-2-yl)ethane-1,2-diamine, 8g

Acetamide 7g (560 mg, 1.29 mmol) in dry dichloromethane (5 mL) was stirred at 0-5 °C for 15 min under argon atmosphere. Recently distilled trimethylsilyl chloride (168 μL, 1.55 mmol) was then added at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH₄ (69 mg, 1.81 mmol) in a small quantity of THF was added at -10-0°C and the stirring continued for 2.5 h at the same temperature. The mixture was then treated with NaOH 10%, the resulting inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude product was dissolved in dichloromethane and washed with brine. After the evaporation of the solvent, the crude product was purified through column chromatography using ether:n-hexane (1:1) and CHCl₃:MeOH (9:1), as system solvents to afford diamine 8f as a pale yellow oil; yield 141 mg (26%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 1.45 (d, J = 12Hz, 2H, 4eq,9eq-adamantyl), 1.59 (s, 3H, 8-geranyl), 1.63 (s, 3H, 7-geranyl CH₃), 1.67 (s, 3H, 3-geranyl CH₃), 1.59-1.75 (m, 6H, 1,3,5,7,8eq,10eq-adamantyl), 1.81 (s, 1H, 6eq-adamantyl), 1.95 (s, 1H, 6ax-adamantyl), 1.99-2.10 (m, 4H, 4,5-geranyl), 2.18-2.23 (m, 4H, 4ax,8ax,9ax,10ax-adamantyl ), 2.75 (t, J = 6Hz, 2H, NHCH₂CH₂NH-geranyl), 2.84 (t, J = 6 Hz, 2H, NHCH₂CH₂NH-geranyl), 2.99 (s, 2H, benzyl), 3.32 (d, J = 7 Hz, 2H, 1-geranyl), 5.07 (t, J = 7 Hz, 1H, 6-geranyl), 5.26 (t, J = 7 Hz, 1H, 2-geranyl), 7.16-7.30 (m, 5H, phenyl); ¹³C NMR (CDCl₃, 100MHz) δ (ppm) 16.8 (3-geranyl CH₃), 18.0 (8-geranyl), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl), 27.8
(5-adamantyl), 28.3 (7-adamantyl), 33.1 (4,9-adamantyl), 34.0 (8,10-adamantyl), 34.4 (1,3-adamantyl), 37.3 (benzyl), 38.8 (NH\(_2\)CH\(_2\)CH\(_2\)NH-geranyl), 39.3 (6-adamantyl), 39.9 (4-geranyl), 46.9 (1-geranyl), 49.3 (NH\(_2\)CH\(_2\)CH\(_2\)NH-geranyl), 59.0 (2-adamantyl), 124.2 (6-geranyl), 126.4 (phenyl), 128.5 (phenyl), 130.5 (phenyl), 132.0 (7-geranyl), 138.7 (quaternary-phenyl); HRMS (ESI-TOF (+)) m/z [M + H]\(^+\) calculated for \([C_{29}H_{45}N_2]^+\) 421.3577, found 421.3571; Purity of the product determined by HPLC-MS: 98.6%.

**2-((3,7-Dimethyl-octa-2,6-dien-1-yl)amino)-N-(2-phenyladamantan-2-yl)acetamide, 7h**

Bromoacetamide 6h (1.17 g, 3.36 mmol) in dry THF (20 mL) was added dropwise at 0 °C to a stirred solution of geranylamine (2) (514 mg, 3.36 mmol) and triethylamine (340 mg, 3.36 mmol) in dry THF (30 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl\(_3\):MeOH (9:1), as eluents. Acetamide 7h was obtained as a yellow oil; yield 920 mg (35%); \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \(\delta\) (ppm) 1.54 (s, 3H, 8-geranyl), 1.60 (s, 3H, 7-geranyl CH\(_3\)), 1.68 (s, 3H, 3-geranyl CH\(_3\)), 1.68-2.18 (m, 18H, 1,3,4,5,6,7,8,9,10-adamantyl, 4,5-geranyl), 3.07 (d, \(J = 7.4\) Hz, 2H, 1-geranyl), 3.13 (s, 2H, COCH\(_3\)NH), 5.07 (t, \(J = 7.4\) Hz, 1H, 6-geranyl), 5.14 (t, \(J = 7.4\) Hz, 1H, 2-geranyl), 7.17-7.33 (m, 3H, phenyl), 7.58-7.59 (m, 2H, phenyl); \(^{13}\)C-NMR (CDCl\(_3\), 100MHz) \(\delta\) (ppm) 16.6 (3-geranyl CH\(_3\)), 18.0 (8-geranyl), 26.0 (7-geranyl CH\(_3\)), 26.8 (5-geranyl), 27.0 (5-adamantyl), 27.8 (7-adamantyl), 32.8 (4,9-adamantyl), 33.7 (1,3,8,10-adamantyl), 38.2 (6-adamantyl), 39.9 (4-geranyl), 47.4 (1-geranyl), 52.6 (COCH\(_2\)NH), 61.0 (2-adamantyl), 122.1 (2-geranyl), 124.3 (6-geranyl), 126.7 (phenyl), 128.3 (phenyl), 132.0 (7-geranyl), 139.3 (quaternary-phenyl), 143.5 (3-geranyl), 169.8 (C=O); HRMS (ESI-TOF (+)) m/z [M + H]\(^+\) calculated for \([C_{28}H_{41}N_2O]^+\) 421.3213, found 421.3214; Purity of the product determined by HPLC-MS: 97.8%.

**N-(3,7-dimethyl-octa-2,6-dien-1-yl)-N'-(2-phenyl-adamantan-2-yl)ethane-1,2-diamine, 8h**
Acetamide 7h (800 mg, 1.90 mmol) in dry dichloromethane (10 mL) was stirred at 0-5 °C for 15 min under argon atmosphere. Recently distilled trimethylsilyl chloride (247 μL, 2.28 mmol) was then added at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH₄ (101 mg, 2.66 mmol) in a small quantity of THF was added at -10-0°C and the stirring continued for 2.5 h at the same temperature. The mixture was then treated with NaOH 10%, the resulting inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude product was dissolved in dichloromethane and washed with brine. After the evaporation of the solvent, the crude product was purified through column chromatography using ether:n-hexane (1:1) and CHCl₃:MeOH (9:1), as system solvents to afford the diamine 8h as a pale yellow oil; yield 290 mg (36%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 1.56 (s, 3H, 8-geranyl), 1.59 (s, 3H, 7-geranyl CH₃), 1.67 (s, 3H, 3-geranyl CH₃), 1.67-1.75 (m, 9H, 1,3,4eq,5,6eq,7,8eq,9eq,10eq-adamantyl), 1.89 (s, 1H, 6ax-adamantyl), 1.98-2.09 (m, 4H, 4,5-geranyl), 2.40 (d, J = 12.3Hz, 2H, 8ax,10ax-adamantyl), 2.23 (t, J = 6Hz, 2H, NHCH₂CH₂NH-geranyl), 2.48-2.51 (m, 4H, 4ax,9ax-adamantyl, NHCH₂CH₂NH-geranyl), 3.05 (d, J = 7.0 Hz, 2H, 1-geranyl), 5.07 (t, J = 7 Hz, 1H, 6-geranyl), 5.15 (t, J = 7 Hz, 1H, 2-geranyl), 7.16-7.20 (m, 1H, phenyl), 7.30-7.37 (m, 4H, phenyl); ¹³C NMR (CDCl₃, 400MHz) δ (ppm) 16.6 (3-geranyl CH₃), 18.0 (8-geranyl), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl C), 27.4 (5-adamantyl), 28.4 (7-adamantyl), 33.0 (4,9-adamantyl), 33.2 (1,3-adamantyl), 34.4 (8,10-adamantyl), 38.5 (6-adamantyl), 39.9 (4-geranyl), 46.7 (1-geranyl), 49.4 (NHCH₂CH₂NH-geranyl), 61.3 (NHCH₂CH₂NH-geranyl), 66.2 (2-adamantyl), 122.2 (2-geranyl), 124.4 (6-geranyl), 126.2 (phenyl), 126.6 (phenyl), 128.4 (phenyl), 131.9 (7-geranyl), 138.8 (quaternary-phenyl), 145.0 (3-geranyl); HRMS (ESI-TOF (+)) m/z [M + H]^+ calculated for [C₂₈H₄₃N₂]^+ 407.3421, found 407.3414; Purity of the product determined by HPLC-MS: 97.0%.

2-((3,7-Dimethylocta-2,6-dien-1-yl)amino)-N-(2-(5-phenylthiazol-2-yl)adamantan-2-yl)acetamide, 7i
Bromoacetamide 6i (414 mg, 0.96 mmol) in dry THF (6 mL) was added dropwise at 0 °C to a stirred solution of geranylamine (2) (147 mg, 0.96 mmol) and triethylamine (97 mg, 0.96 mmol) in dry THF (8.5 mL). The stirring continued for 48 h at room temperature. Then the aqueous phase was extracted twice with dichloromethane, the combined organic extracts were evaporated in vacuo and the crude product was purified through column chromatography using a. ether:n-hexane (1:1), b. CHCl₃:MeOH (9:1), as eluents. Acetamide 7i was obtained as a pale yellow oil; yield 400 mg (83%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 1.55 (s, 3H, 8-geranyl), 1.59 (s, 3H, 7-geranyl CH₃), 1.67 (s, 3H, 3-geranyl CH₃), 1.54-1.83 (m, 7H, 4eq,5,6eq,7,8eq,9eq,10eq-adamantyl), 1.86-1.99 (s, 3H, 6ax,8ax,10ax-adamantyl), 2.02-2.17 (m, 6H, 4ax,9ax-adamantyl, 4,5-geranyl), 3.12 (s, 2H, COCH₂NH), 3.14 (s, 1H, 1,3-adamantyl), 3.16 (m, 2H, 1-geranyl), 5.05 (m, 1H, 6-geranyl), 5.16 (m, 1H, 2-geranyl), 7.27-7.31 (m, 1H, phenyl), 7.36-7.42 (m, 3H, phenyl, thiazolyl) 7.85-7.90 (m, 2H, phenyl); ¹³C-NMR (CDCl₃, 100MHz) δ (ppm) 16.6 (3-geranyl CH₃), 18.0 (8-geranyl), 26.0 (7-geranyl CH₃), 26.8 (5-geranyl), 27.3 (5-adamantyl), 27.4 (7-adamantyl), 33.6 (4,9-adamantyl), 33.9 (8,10-adamantyl), 35.0 (1,3-adamantyl), 38.2 (6-adamantyl), 39.9 (4-geranyl), 47.4 (1-geranyl), 52.5 (COCH₂NH), 59.0 (2-adamantyl), 113.1 (phenyl), 124.2 (2-geranyl), 126.6 (phenyl), 128.0 (thiazolyl), 128.9 (phenyl), 132.0 (6-geranyl), 135.3 (7-geranyl), 153.7 (3-geranyl), 168.9 (C=O), 170.2 (quaternary-C thiazolyl), 174.6 (quaternary-C thiazolyl); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₃₁H₄₂N₃OS]⁺ 504.3043, found 504.3054.

N-(3,7-dimethylocta-2,6-dien-1-yl)-N’-(2-(5-phenylthiazol-2-yl)adamantan-2-yl)ethane-1,2-diamine, 8i
Acetamide 7f (400 mg, 0.79 mmol) in dry dichloromethane (4 mL) was stirred at 0-5 °C for 15 min under argon atmosphere. Recently distilled trimethylsilyl chloride (103 μL, 0.95 mmol) was then added at the same temperature and the mixture was stirred for another 15 min. A suspension of LiAlH₄ (42 mg, 1.11 mmol) in a small quantity of THF was added at -10-0 °C and the stirring continued for 2.5 h at the same temperature. The mixture was then treated with NaOH 10%, the resulting inorganic precipitate was filtered off, the organic phase was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic extracts were evaporated in vacuo and the crude was dissolved in dichloromethane and washed with brine. After the evaporation of the solvent, the crude product was purified through column chromatography using ether:n-hexane (1:1) and CHCl₃:MeOH:NH₃ (88:10:2), as system solvents to afford diamine 8i as a pale yellow oil; yield 120 mg (31%); ¹H-NMR (CDCl₃, 400 MHz) δ (ppm) 1.54 (s, 3H, 8-geranyl), 1.57 (s, 3H, 7-geranyl CH₃), 1.65 (s, 3H, 3-geranyl CH₃), 1.67-1.79 (m, 7H, 4eq,5,6eq,7eq,9eq,10eq-adamantyl), 1.92 (s, 1H, 6ax-adamantyl), 1.99-2.06 (m, 6H, 8ax,10ax-adamantyl, 4,5-geranyl), 2.35-2.38 (d, J = 13.8 Hz, 2H, 4ax,9ax-adamantyl), 2.47 (s, 2H, 1,3-adamantyl), 2.61 (t, J = 5.5 Hz, 2H, NHCH₂CH₂NH-geranyl), 2.70 (t, J = 5.5 Hz, 2H, NHCH₂CH₂NH-geranyl), 3.29 (d, J = 7.6 Hz, 2H, 1-geranyl), 5.02 (t, J = 7.6 Hz, 1H, 6-geranyl), 5.24 (t, J = 7.6 Hz, 1H, 2-geranyl), 7.28-7.33 (m, 1H, phenyl), 7.38-7.42 (m, 3H, phenyl, thiazolyl), 7.89 (d, J = 6.2 Hz, 2H, phenyl); ¹³C NMR (CDCl₃, 400MHz) δ (ppm) 16.7 (3-geranyl CH₃), 18.0 (8-geranyl), 26.0 (7-geranyl CH₃), 26.6 (5-geranyl), 27.6 (5-adamantyl), 27.8 (7-adamantyl), 33.0 (4,9-adamantyl), 34.6 (8,10-adamantyl), 35.7 (1,3-adamantyl), 38.5 (6-adamantyl), 39.9 (4-geranyl), 45.1 (1-geranyl), 47.3 (NHCH₂CH₂NH-geranyl), 64.0 (NHCH₂CH₂NH-geranyl), 66.2 (2-adamantyl), 112.5 (phenyl), 124.0 (2-geranyl), 126.6 (phenyl), 128.2 (thiazolyl), 129.0 (phenyl), 132.2 (6-C), 135.1 (7-C), 154.0 (3-C), 178.1 (quaternary-C thiazolyl); HRMS (ESI-TOF (+)) m/z [M + H]⁺ calculated for [C₃₁H₄₄N₃S]⁺ 490.3250, found 490.3253. Purity of the product determined by HPLC-MS: 99.3%.

**Differential scanning calorimetry.** Samples for DSC were prepared by mixing the tested ligands SQ109 (8a) and analogs 8b-j, 12 with DMPC or DSPG membranes at 10% w/w concentration. This was achieved by mixing a solution of the ligand’s fumarate salt in methanol and a solution of DMPC lipid in chloroform or a solution of DSPC in chloroform/methanol (5:1) and subsequently evaporating the solvent. The films that were formed were utilized for DSC analysis, by weighting approximately 3 mg of each film in a 40 μL aluminum crucible, adding 3 μL of PBS (pH = 7.4) as hydration medium, sealing the crucible, vortexing and allowing it to anneal for a 15 min period, in order to achieve sample
equilibration. The DSC thermogram of each sample was obtained by utilizing a DSC822° Mettler-Toledo calorimeter (Schwerzenbach, Switzerland), calibrated with pure indium ($T_m = 156.6 \ ^\circ C$). An empty sealed crucible was used as reference. Each analysis included equilibration at 5 °C for 10 min, followed by two heating-cooling cycles in the range between 5-35 °C with a 5 °C/min scanning rate for DMPC samples or between 10-70 °C, with a 2.5 °C/min scanning rate for DSPG samples. The obtained calorimetric data (characteristic transition temperatures $T_{onset}$ and $T_r$, transition enthalpy $\Delta H$ and width at half peak height of the $C_p$ profiles $\Delta T_{1/2}$) were analyzed with the Mettler-Toledo STAR® software. The transition enthalpy is expressed as joules per total sample mass and as kilojoules per moles of DMPC and is presented as absolute value in endothermic and exothermic processes (Table 5). LogD values in Table 5 were calculated with ChemAxon software.

**Cell growth inhibition assays.** Cell growth inhibition assays were performed as previously reported$^{24, 47, 48}$ are described in the Supporting Information including representative dose-response curves of SQ109 analogs against the protozoan parasites (Figure S1).

**Surface Plasmon Resonance.** The MtMmpL3 protein was purified from *M. smegmatis* DmmpL3/pMVGH1-mmpL3tb cells$^{49}$ following the previously reported protocol.$^{10}$ For the amino coupling of MmpL3tb, CM5 chip surfaces were activated with 0.05 M N-hydroxysuccinimide and 0.2 M N-ethyl-N-(3-dieethylaminopropyl)carbodiimide (BIAcore). Purified MmpL3tb was injected over surfaces immediately after activation. The immobilized density of the protein (ligand) was 17,654 response units (RU). For kinetic modeling, we considered only the simplest models that would be compatible with one or two distinct events during both inhibitor binding and dissociation. These four models are (i) simple 1:1 binding model; (ii) heterogeneous ligand (HL), in which different protein populations on-chip surface have different kinetic properties; (iii) two-state reaction or ligand-induced conformational change, wherein conformational change occurs on the same time scale as ligand binding (Figure S2); and (iv) bivalent analyte, where multiple analytes bind independently at nonidentical sites.$^{10, 50}$

**ASSOCIATED CONTENT**

**Supporting Information.**
The Supporting Information is available free of charge at [https://pubs.acs.org/doi/XXXX](https://pubs.acs.org/doi/XXXX) DSC scans and results for the cooling process of selected SQ109 analogs (one Figure and one Table), A Figure for dose-response curves from biological testing, a Figure with curves from SPR, a Figure with DSC cooling scans, Figures with NMR spectra and HPLC plots. Supplementary information for
methods and protocols for synthetic intermediates, biological assays and Supporting Information references.

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Author Contributions
M.S. and S.R.M. contributed equally.

This research is part of MS Ph.D thesis. ADK conceived the project. ADK, MS designed, and MS synthesized and characterized the compounds. MM and SOB in HIZ lab performed binding assays. SRM and ZG performed testing against *M. smegmatis*, *B. subtilis* and *E. coli* in EO lab. AR-C, MV-D in GB lab performed testing against *L. mexicana*. NN and MC ran the DSC experiments, reported and interpreted the results in CD lab. A-LT performed the HRMS experiments and interpreted the results with SV. K-HB, T-NP, HL and JHN performed testing against *T. brucei*, *T. cruzi* and *L. donovani*. Alcohol 5i was produced by NE and IPP. IS ran the DFT calculations for SQ109 conformers in ADK lab. MHC designed and CMS performed testing against MtErdman and MtH37Rv. NA-C and TD determined activity against Mtb HN878. SW, MvdW and DC performed testing against *P. falciparum* parasites and HepG2 assays in L-MB lab. LK conceived experiments and analyzed data. MA performed experiments and analyzed data. JB carried out microsomal stability and solubility studies and wrote the results with SV. ADK, EO, MS, and SRM interpreted results and wrote the manuscript with all co-authors involved in the final editing of the paper.

**Abbreviations Used**

Bs, *Bacillus subtilis*; cryo-EM, cryogenic electron microscopy, DSC, differential scanning calorimetry; DEAD, diethyl azodicarboxylate; DFT, Density Functional Theory; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DSPG, 1,2-distearoyl-sn-glycero-3-phosphoglycerol; Ec, *Escherichia coli*; esp = α,α,α′,α′-tetramethyl-1,3-benzenedipropionic acid; GAFF, Generalized Amber Force Field; HepG2, hepatocyte carcinoma; HOSA, hydroxylamine-O-sulfonic acid; HFIP, hexafluoroisopropanol; HRMS, High-resolution mass spectrometry; TB, Tuberculosis; *Mtb*, *Mycobacterium tuberculosis*; MDR, multidrug-resistant; XDR, extensively drug-resistant; MmpL3, mycobacterium membrane protein large 3; Ms, *Mycobacterium smegmatis*; MtE, *M. tuberculosis* Erdman; MtH878, *M. tuberculosis* HN878; OPM, Orientations of Proteins in Membranes; PBS, phosphate buffer solution; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; RMSD, root mean square deviation; TMM, trehalose monomycolate; PMF, Proton motive force; RMSD, root-mean-square deviation; rt, room temperature; SID, simulation interaction diagram; THF, tetrahydrofuran; *T. brucei*, *Trypanosoma brucei*; *T. cruzi*, *Trypanosoma cruzi*.

**Notes**

The authors declare no competing financial interest.
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