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Novel polycyclic 'cage'-1,2-diamines as potential anti-tuberculosis agents

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ABSTRACT

A series of polycyclic 'cage' derivatives of *N*-geranyl-1,2 diamines were synthesized and screened for their anti-mycobacterial activity against $H_{37}Rv$, multidrug resistant (MDR) and extensively drug-resistant (XDR) strains of tuberculosis. By substituting the adamantyl skeleton of SQ109 with trishomocubanyl (**9**), oxa-pentacycloundecyl (**14**, **16**), pentacycloundecyl, PCU, (**10**, **15**) and azapentacycloundecyl (**22**, **23**), the effect of other polycyclic "cage" skeletons could be investigated. Compound **9** (trishomocubanyl moiety) proved to be the most active (MICs: 0.5–2 μg/mL) while PCU hydroxyl derivatives (**15** and **23**), oxa-pentacycloundecyl and azapentacycloundecyl derivatives displayed similar activity to SQ109 (MICs: 0.5–4 μg/mL) against all three strains of TB used in this study.

1. Introduction

Tuberculosis (TB) is a highly contagious and insidious disease with a high infection rate that has been present in humans since antiquity. TB is predominantly caused by *Mycobacterium tuberculosis* which is a slow growing bacterium. The causal organism can remain dormant in the host (human) for a very long time and may only become active when the person falls sick or has a back drop in his/her immune system. This event in particular has become most prominent in immuno-compromised patients such as those living with HIV (Human Immunodeficiency Virus). The 2011 global tuberculosis control report of the World Health Organisation (WHO), estimated that about 8.8 million incident cases of TB occurred globally. An estimated 1.1 million (13%) patients are living with HIV, and 82% of TB cases among people living with HIV occurred in the African region [1].

An urgent need for highly potent, more effective drugs with fewer or no side effects and shorter treatment periods to combat the increasing TB pandemic is therefore apparent. Potential anti-TB drug candidates such as diarylquinolone (TMC207), nitroimidazole (OPC67683 and PA824), pyrrole (LL3858), diamine (SQ109) are in different stages of clinical trials [2–5].

SQ109 (**2**) first reported by Lee et al. [6] shares the same 1,2 ethylenediamine pharmacophore with ethambutol (**1**). SQ109 possesses remarkable activity against MDR-TB which includes the EMB resistant strain suggesting that SQ109 is an anti-TB agent with a new mechanism [7]. SQ109 induces a synergistic effect when used in combination with other first line drugs such as isoniazid and rifampicin, however an additive effect is observed when used with streptomycin [8].

The incorporation of polycyclic "cage" compounds (such as adamantane and pentacycloundecane, PCU) into drugs with pharmaceutical applications has enjoyed much attention from researchers for several decades starting with the discovery of amantadine, an anti-viral drug [9–11]. Polycyclic "cage" compounds have the ability to improve drug lipophilicity, thus serving as a transport aid in carrying such drugs across cell membranes. It has been reported that 'cage' moieties such as adamantane and PCU are able to cross the Blood Brain Barrier (BBB) and the Central Nervous System (CNS) [9,12,13]. It also helps to reduce the bio-degradation of drugs in biological systems thus prolonging their pharmaceutical effect in the body [9,14,15]. SQ109 (**2**) and SQ117 (**3**) both have a lipophilic moiety, namely the 2-adamantyl moiety in SQ109 (log *P*; 5.26) and a diphenyl moiety in SQ117 (log *P*; 5.50). However, different MIC values were reported

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for each [7]. SQ109 was five-fold more active than SQ117 suggesting that the nature of the lipophilic group does play a role in their activities even though SQ117 possesses a higher log *P* value [7] Fig. 1.

In the course of our research for novel anti-tubercular compounds [16,17], we recently demonstrated the importance of the length, saturation and di-substitution of the alkene chain of some SQ109 derivatives [17,18]. This present study aims at investigating the possibility of further enhancing/improving the anti-TB activity of the diamine *via* substitution of the adamantyl moiety with other polycyclic compounds such as trishomocubane and pentacycloundecane. Based on this; seven novel diamine based compounds bearing the lipophilic pentacycloundecane, oxa-pentacycloundecane aza-pentacycloundecane and trishomocubane cages were synthesized and screened for activity against drug sensitive ($H_{37}Rv$) and drug-resistant strains of tuberculosis.

2. Results and discussion

2.1. Chemistry

Cookson's dione **4** [19,20] was the starting material for the synthesis of trishomocubanone **5** and the PCU-monoketone **6**. Trishomocubanone was synthesized *via* a six step reaction pathway [21,22] and PCU monoketone was synthesized *via* a five step reaction pathway as reported in literature [21–23] (Scheme 1). Geranyl bromide **7** was reacted with excess ethane-1,2-diamine in dry dichloromethane at −78 °C to afford (*E*)-*N*′-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine **8** (86% yield). Isoprenyl ethane-1,2-diamine **8** was reacted with trishomocubanone, PCU monoketone and 2-adamantanone *via* reductive amination; the resulting imines were reduced with $NaBH_4$ to obtain polycyclic-diamines; the diamines were converted to their HCl salts to obtain compounds **9** [*N*-geranyl-*N*′-(11-trishomocubanyl)ethane-1,2-diamine hydrochloride] and SQ109 [*N*-geranyl-*N*′-(2-adamantyl)ethane-1,2-diamine hydrochloride] (55–57% yield). The reaction with PCU monoketone to yield **10** [*N*-geranyl-*N*′-(8-pentacycloundecyl) ethane-1,2-diamine hydrochloride] was successful. The expected orientation of the nitrogen atom on the PCU skeleton upon reductive amination with $NaBH_4$ is the *endo*-form (**10**) [24,25]. This orientation was confirmed by a NOESY experiment as H-8 (2.67 ppm) displayed through space interaction with H-5 and H-9 (2.16–2.18 ppm) Scheme 2.

It was anticipated that the presence of a hydroxyl group (*endo*/*exo* positioning) on the cage moiety might contribute significantly to its activity; this led to the design and successful synthesis of compound **15** and **23**. 8-Benzylamino-8, 11-oxapentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane **11** was synthesized as reported in literature [13,26]. Compound **11** was debenzylated using 10% Pd/C under hydrogen gas at atmospheric pressure to obtain **12** in 60.8% yield [27]. Compound **12** was reacted with chloroacetyl chloride to obtain compound **13** (62% yield), which was reacted with geranylamine [17] to obtain compound **14** (58.5% yield). Compound **14** was reduced with a strong agent (LAH) at a 1:4 ratio in dry THF under reflux conditions to obtain a compound with *m*/*z* of 357.2897 ($M + H^+$, 33.7% yield). Compound **15** was proposed and this structure was confirmed using 2D NMR experiments. A through space interaction of H-11 (3.86 ppm) with H-1 (2.68 ppm), H-3 (2.30 ppm) and H-10 (2.36 ppm) was observed thus proving that the hydroxyl group was at an *endo* position. H-8 also displayed a NOESY interaction with H-5 (2.34 ppm), H-7 (2.80 ppm), H-9 (2.51 ppm) and H-1′/2′ (ethylene protons at 3.18 ppm) while the methylene protons, H-1′/2′, displayed NOESY interactions with methyl protons (1.61 ppm), H-7, H-8 and H-9 respectively. These assignments confirm the *endo* positioning of the isoprenyl diamine moiety on the PCU cage as a result of the opening of the ether bridge.

Attempts to synthesize the H-8 *endo*-orientated compound **10** prove unsuccessful; however an *exo* positioning of the R-group would be obtained if an oxa-pentacycloundecyl moiety is used, this led to the design and successful synthesis of compound **16**. This was achieved by using a milder reducing reagent (65% Red-Al in toluene) on compound **14** to obtain **16** (45.2% yield). NMR spectroscopic analysis of compound **16** showed the successful synthesis, as no extra methine carbon was observed at position 57.4 ppm and a quaternary carbon (C-8) at position 110.1 ppm was observed. C-8 displays a HMBC correlation with methylene protons (H-1′) at 3.02 ppm while H-11 (4.58 ppm) also displayed NOESY interactions with H-1, H-3 and H-10 respectively, thus confirming the successful synthesis of compound **16** Scheme 3.

Replacement of the oxo-bridge of **16** with an aza-bridge to give the isomeric hemiaminal **23** was also achieved (Scheme 4) and this compound was screened for anti-TB activity. Mono-protection of Cookson's diketone **4** was carried out to obtain the ethylene ketal **17** in 74% yield and condensation with benzylamine gave the imine **18**. Reduction with $NaBH_4$, followed by hydrolysis resulted in the formation of the racemic hexacyclic cage amine **19**. Benzyl deprotection of **19** led to compound **20** (44% yield). Geranylamine was reacted with chloroacetyl chloride to afford (*E*)-2-chloro-*N*-(3,7-dimethylocta-2,6-dienyl)acetamide (**21**, 81% yield) which was reacted with 4-azahexacyclo [5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}] dodecan-3-ol (**20**) to obtain **22** (81% yield). Reduction of **22** to yield the desired compound **23** (72% yield) was achieved using 65% Red-Al in toluene.

The successful syntheses of all novel compounds were confirmed using ^1H, ^{13}C, 2D NMR experiments (COSY, HSQC, HMBC, ROESY and NOESY), IR and HR-MS.

2.2. Anti-tubercular activity

All compounds (except SQ109 which is achiral) were tested as a racemate. The *in vitro* anti-mycobacterial activity of all the compounds against *M. tuberculosis* was carried out using the Mycobacteria Growth Indicator Tube system (MGIT). The minimum inhibitory concentration (MIC, μg/mL) was detected by BACTEC 960TB (Becton Dickinson). All compounds were screened against $H_{37}Rv$ (ATCC No: 25177), MDR and XDR strains of tuberculosis and the results are summarized in Table 1. Compounds **14** and **22** did not show any promising anti-TB activity implying that the carbonyl group contributes negatively to their inhibitory effect. Compounds **15** (*endo*-positioned hydroxyl group) and **23** (*exo*-positioned hydroxyl group) displayed similar activity as SQ109 against both MDR and XDR strains of TB at a MIC of 2 and 4 μg/mL

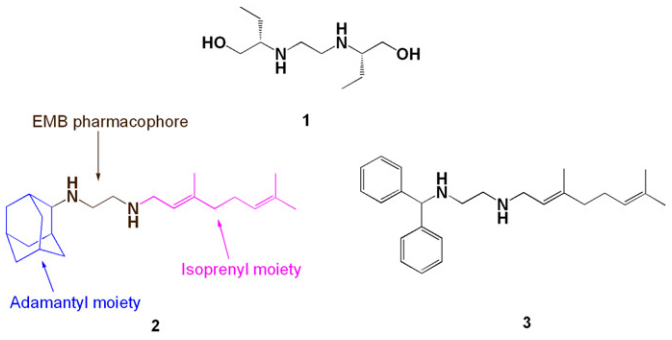
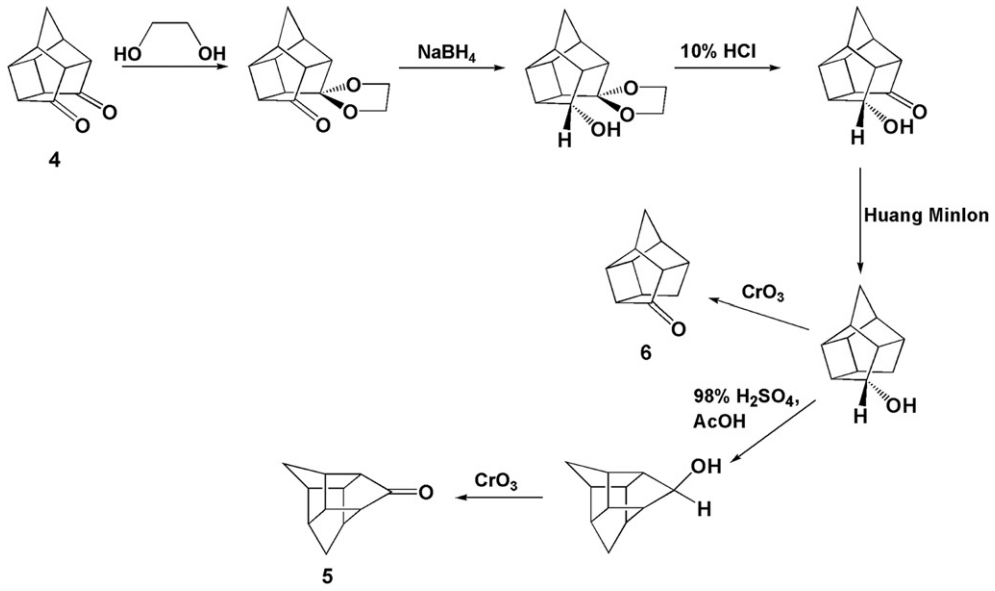


Fig. 1. Structure of ethambutol (**1**), SQ109 (**2**) and SQ117 (**3**).



Scheme 1. Synthetic route for the synthesis of trishomocubanone **5** and the PCU monoketone **6**.

respectively. On the other hand, compound **10** (similar to **15**) without the hydroxyl group displayed reduced activity against all tested TB stains; it appears that the hydroxyl group not withstanding its positioning is essential for anti-tubercular activity. The oxa-pentacycloundecyl derivative **16** proved to be more active (two-fold) than **10** suggesting that the *exo* positioning of the isoprenyl diamine moiety on the pentacycloundecanyl moiety is essential for the anti-TB activity. Compound **9** proves to be the most active in this series; with a twofold increase in activity against MDR and XDR-TB when compared to **10**, **15**, **16**, **23** and SQ109. The D_3-trishomocubyl derivative **9** displayed significantly higher activity than its pentacycloundecyl and adamantyl counterparts.

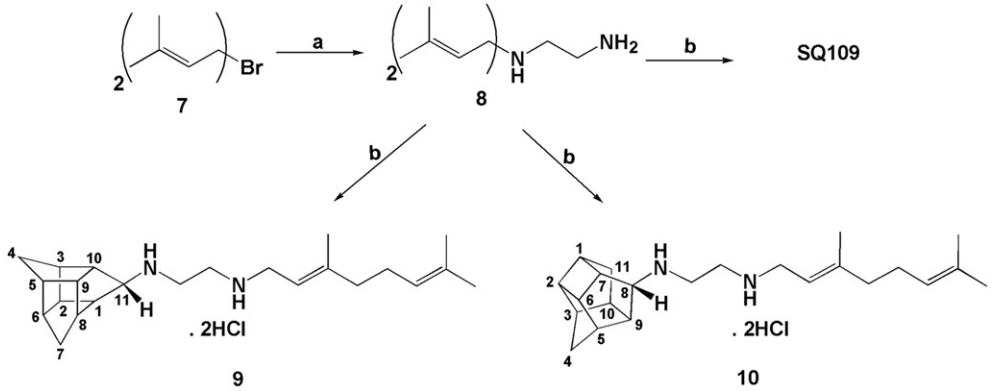
This result suggests that the nature or type of polycyclic "cage" compounds is important for activity. As observed by some researchers in the field [9,11] the substitution of the polycyclic "cage" moiety (adamantyl) with similar moieties such as trishomocubane, pentacycloundecane, oxa-pentacycloundecane *etc*. in most cases maintains the activity of such compounds. Of all reported polycyclic 'cage' compounds only trishomocubane have a unique D_3 stereochemistry which could have contributed to its increased activity in this series.

3. Conclusion

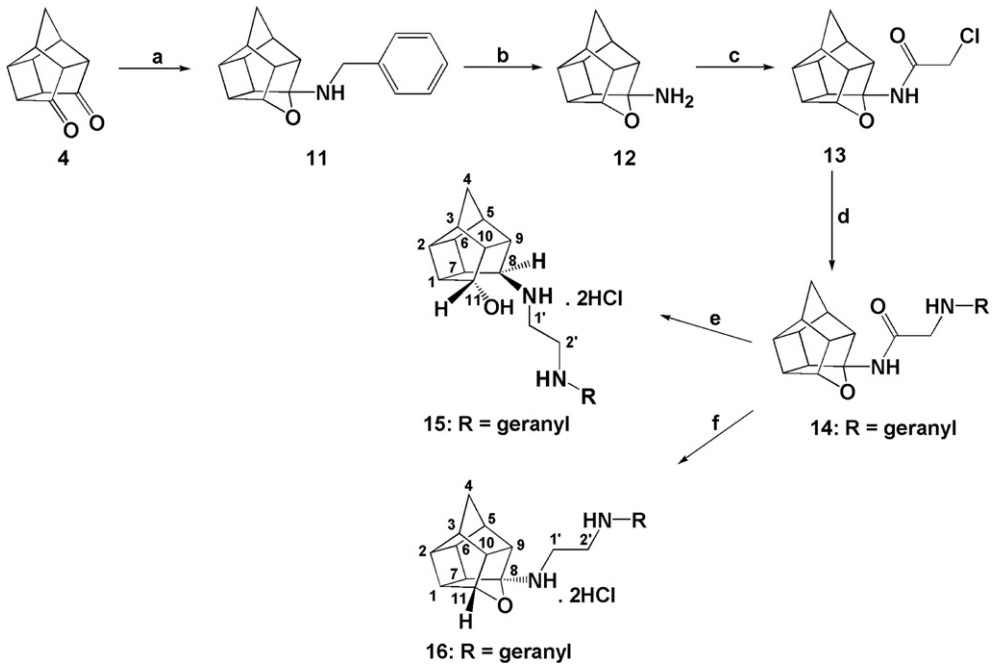
We have synthesized a series of novel polycyclic 'cage' diamine derivatives with potent anti-TB activity. As observed, the positioning of the isoprenyl diamine on the PCU moiety either *endo* or *exo* does influence its anti-TB activity while the hydroxyl group is also essential for efficacy. Compound **9** (trishomocubyl moiety) was identified as the most potent against MDR and XDR strains of TB used with a two-fold increase in activity than **10**, **15** (pentacycloundecyl), **16** (oxa-pentacycloundecyl), **23** (azapentacycloundecyl), and SQ109 (2-adamantyl). This suggests that the type of polycyclic 'cage' moiety used has an influence on the activity of this compound. Further studies are ongoing in our laboratory to derivatise the lead compound with the possibility of enhancing its anti-TB activity.

4. Experimental

The NMR spectroscopic data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments using $CDCl_3$ as a solvent. All chemical shifts (δ) were quoted in parts per million downfield from TMS and the coupling constants (J) recorded in Hertz. Splitting

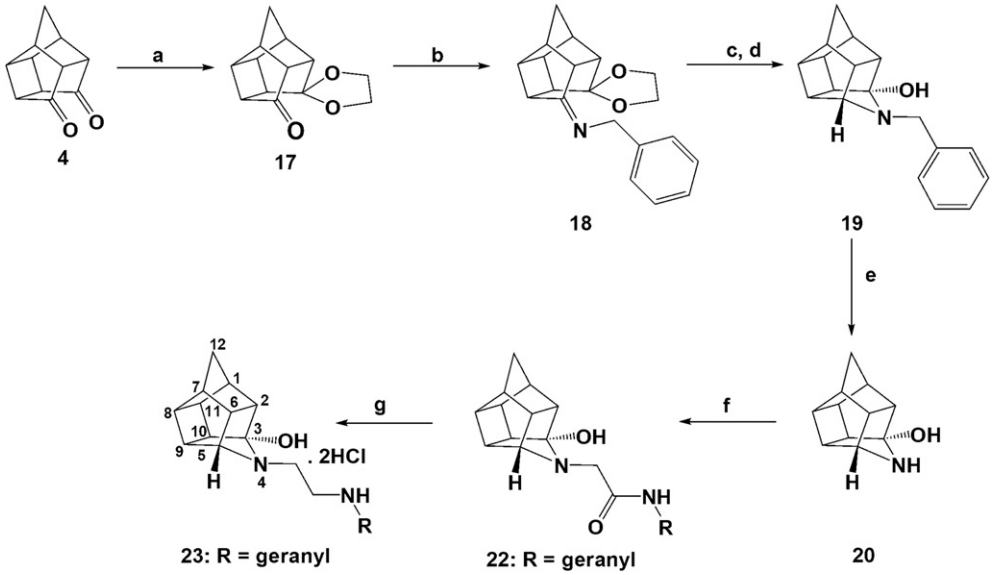


Scheme 2. Reagents and conditions: (a) ethylene diamine (100:1), −78 °C, dry DCM; (b) MeOH, polycyclic 'cage' monoketone (1.2:1), N_2 atmosphere, 2 h, $NaBH_4$, overnight; then HCl, MeOH.



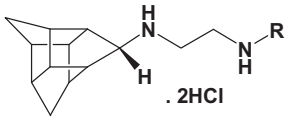
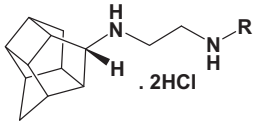
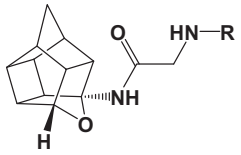
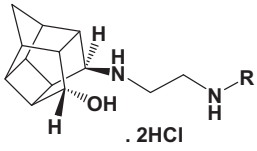
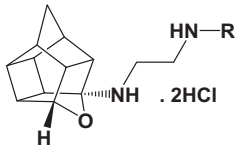
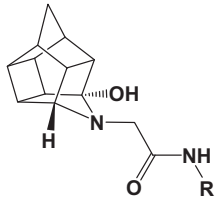
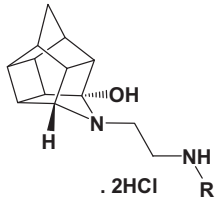
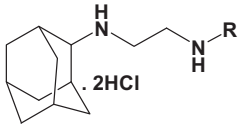
Scheme 3. Reagents and conditions: (a) benzylamine, THF, 0 °C–5 °C, 20 min; azeotropic distillation, benzene, 1 h, $NaBH_4$, 24 h; (b) 10% Pd/C (1:1) mass ratio, H_2 gas, atmospheric pressure; (c) chloroacetyl chloride, dry DCM, K_2CO_3, reflux for 12 h; (d) geranylamine (1:2) mole ratio, K_2CO_3, dry THF, reflux; (e) LAH (1:5) mole ratio, dry THF, reflux, N_2 atmosphere, 12 h; then HCl, MeOH. (f) Red-Al (1:5) mole ratio, dry THF, reflux, N_2 atmosphere; then HCl, MeOH.

pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Reactions were monitored using thin layer chromatography (TLC, Merck Kieselgel 60, F254). Most purifications were achieved with column chromatography using Fluka Kieselgel 60 (70–230 mesh) and $CHCl_3$:CH_3OH:NH_4OH (88:10:2) as the eluent (solvent mixture). Purification of compound **15** were done *via* semi-preparative HPLC on a Shimadzu, LC-6AD instrument using water (solvent A) and acetonitrile (solvent B) while methanol (as Solvent B) was used for compound **23**. An ACE 5C18 150 × 21.2 mm column was used. A gradient elution system of 95% solution A and 5% solution B which changes linearly over 25 min to 20% solution A and 80% solution B at 15 mL/min, detected on a UV–VIS detector at 215 and 254 nm. Mass Spectra were obtained using a Bruker MicroTOF QII Time of Flight mass spectrometer while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3. Melting points results were uncorrected. Tetrahydrofuran was freshly distilled before use from a flask containing sodium benzophenone under N_2 atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use.



Scheme 4. Reagent and conditions: (a) ethylene glycol, *p*-toluenesulfonic acid (cat.), toluene, reflux, Dean–Stark Conditions; (b) benzylamine, EtOH, 100 °C, 18 h; (c) $NaBH_4$, EtOH, rt, 8 h; (d) acetone, 4 M HCl, 12 h, basified with 1M NaOH; (e) MeOH, 10% Pd/C, H_2 atm; (f) 21, K_2CO_3, THF, reflux, H_2 atm; (g) Red-Al (1:5) mole ratio, dry THF, reflux, N_2 atmosphere; then HCl, MeOH.

Table 1
The MICs of the target compounds against *M. tuberculosis* ($H_{37}Rv$, MDR and XDR) strains.

Compound	Structure	MIC (μg/mL)		
		$H_{37}Rv$	MDR	XDR
9		0.5	1	2
10		1	4	8
14		1	4	8
15		0.5	2	4
16		1	2	4
22		1	4	8
23		1	2	4
SQ109		0.5	2	4

SQ109 was used as the reference drug; R = geranyl moiety. Values reported were done in triplicate.

4.1. Synthesis of (E)-N′-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (**8**) [17]

To a vigorously stirred solution of ethane-1,2-diamine (55.4 g, 0.92 mol) in dichloromethane (400 mL) at −78 °C under N_2 atmosphere was dropwise added geranyl bromide **7** (2 g, 9.2 mmol) in dichloromethane (1200 mL) over 4 h. The reaction mixture was left to attain room temperature with stirring over night. The solution mixture was reduced *in vacuo* to about 500 mL and washed with water to remove excess ethane-1,2 diamine, the organic extract was dried over Mg_2SO_4 and concentrated *in vacuo*. The crude residue was purified using column chromatography [eluent; $CHCl_3$:CH_3OH:NH_4OH (88:10:2)] to give a light yellow oil (1.56 g, 86%, $R_f = 0.45$). ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.36 (NH), 1.56 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.97 (2H, m, CH_2), 2.05 (2H, m, CH_2), 2.63 (2H, t, $J = 5.6$ Hz, CH_2), 2.78 (2H, t, $J = 5.7$, 6.1 Hz, CH_2), 3.21 (2H, d, $J = 6.8$ Hz, CH_2), 5.06 (1H, $J = 6.9$ Hz, CH), 5.23 (1H, $J = 6.8$ Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.3 (CH_3), 17.6 (CH_3), 25.7 (CH_3), 26.5 (CH_2), 39.6 (CH_2), 41.8 (CH_2), 47.1 (CH_2), 52.1 (CH_2), 122.8 (CH), 124.1 (CH), 131.5 (C), 137.7 (C).

4.2. Synthesis of N-geranyl-N′-(trishomocubanyl)ethane-1,2-diamine (**9**), N-geranyl-N′-(pentacycloundecyl)ethane-1,2-diamine (**10**), N-geranyl-N′-(2-adamantyl)ethane-1,2-diamine (SQ109)

A mixture of isoprenyl diamine **8** (1.2 mol) and polycyclic 'cage' monoketone (1.0 mol) in methanol (15 mL) was stirred for 2 h at room temperature under a nitrogen atmosphere. The resulting imine was reduced with solid $NaBH_4$ (1.2 mol) which was added slowly over 15 min and the mixture stirred overnight. Additional methanol (15 mL) was added to the reaction vessel after which water (20 mL) was added to quench excess $NaBH_4$. The solution was extracted with ethyl acetate (2 × 50 mL) and the solution dried over Na_2SO_4 and concentration *in vacuo*. The crude product was purified *via* column chromatography on silica gel using $CHCl_3$:CH_3OH:NH_4OH (88:10:2) as eluent to give a yellow oil and converted to its HCl salt.

4.2.1. Data for N-geranyl-N′-(11-trishomocubyl)ethane-1,2-diamine dihydrochloride (**9**)

A white solid (Mp. 174–178 °C, 0.65 g, yield 57%, $R_f = 0.64$). IR ν_{max}: 3388, 2949, 2871, 2736, 1585, 1443, 1034, 773 and 555 cm^{-1}. MS (TOF) calculated for $C_{23}H_{37}N_2$ ($M + H^+$ of free base) 341.2951, found 341.2940. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.21–1.28 (3H, m, H-4a, H-7a, H-7s), 1.37 (1H, AB, d, $J = 10.2$ Hz, H-4s), 1.53 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.85–1.88 (2H, m, H-1, 2), 1.91–1.96 (4H, m, H-8, 10, CH_2), 1.97–2.03 (5H, m, H-5, 6, 9, CH_2), 2.37 (1H, s, H-3), 2.63–2.69 (4H, m, 2 × CH_2), 2.91 (1H, s, H-11), 3.17 (2H, d, $J = 6.8$ Hz, CH_2), 5.03 (1H, m, CH), 5.19 (1H, m, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.4 (CH_2), 32.8 (C-4), 33.4 (C-7), 39.5 (CH_2), 40.6 (C-8), 41.4 (C-2), 44.2 (C-3), 44.7 (C-9), 46.9 (CH_2), 47.0 (C-5), 47.4 (C-6), 48.0 (CH_2), 49.2 (CH_2), 50.4 (C-10), 51.6 (C-1), 64.2 (C-11), 122.8 (CH), 124.0 (CH), 131.3 (C), 137.5 (C).

4.2.2. Data for N-geranyl-N′-(8-pentacycloundecyl)ethane-1,2-diamine dihydrochloride (**10**)

A white solid (Mp. 158–160 °C, 0.59 g, yield 55%, $R_f = 0.62$). IR ν_{max}: 3140, 3048, 2948, 2694, 1444, 1405, 1034, 793 and 557 cm^{-1}. MS (TOF) calculated for $C_{23}H_{37}N_2$ ($M + H^+$ of free base) 341.2951, found 341.2928. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 0.94 (1H, AB, $J = 11.9$ Hz, H-11a), 1.13 (1H, AB, $J = 10.3$ Hz, H-4a), 1.55 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.64 (1H, s, H-4s), 1.83 (2H, br s, NH), 1.91–1.98 (2H, m, CH_2), 2.02–2.07 (2H, m, CH_2), 2.16–2.18 (3H, m, H-3, 5, 9), 2.26 (1H, s, H-10), 2.31 (1H, AB, $J = 11.7$ Hz, H-11s), 2.43 (1H, m, H-6), 2.49–2.54 (2H, m, H-2, 7), 2.60–2.62 (1H, m, H-1), 2.63–2.70 (5H, m, H-8, 2 × CH_2), 3.19 (2H, *d*, $J = 6.8$ Hz, CH_2), 5.05 (1H, t, $J = 5.6$ Hz, CH), 5.21 (1H, t, $J = 5.9$ Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.4 (CH_2), 28.7 (C-11), 34.6 (C-4), 36.2 (C-1), 37.7 (C-7), 39.6 (CH_2), 40.8 (C-6),

41.8 (C-10), 41.9 (C-2), 44.2 (C-3/5), 44.6 (C-3/5), 46.9 (CH_2), 47.2 (C-9), 48.6 (CH_2), 49.3 (CH_2), 61.9 (C-8), 122.6 (CH), 124.1 (CH), 131.4 (C), 137.7 (C).

4.2.3. Data for SQ109 (N-geranyl-N′-(2-adamantyl)ethane-1,2-diamine) dihydrochloride

A white solid (Mp. 180–184 °C, 0.35 g, 60% yield, $R_f = 0.62$). IR ν_{max}: 3142, 3050, 2910, 2850, 1588, 1459, 1408, 1102, 778 and 553 cm^{-1}. HR-MS calculated for $C_{22}H_{39}N_2$ $(M + H)^+$ 331.3108, found 331.3135; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 1.47 (2H, d, $J = 12.5$ Hz, H-4a/9a), 1.57 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.66–1.68 (4H, m, H-6, 8a/10a), 1.74 (1H, s, H-5), 1.80–1.82 (5H, m, H-1, 3, 7, 8b/10b), 1.95 (2H, d, $J = 12.6$ Hz, H-4b/9b), 1.98–2.00 (2H, m, CH_2), 2.03–2.09 (2H, m, CH_2), 2.67 (1H, s, H-2), 2.70 (4H, s, 2 × CH_2), 3.22 (2H, d, $J = 6.8$ Hz, CH_2), 5.06 (1H, m, CH), 5.24 (1H, t, $J = 6.7$ Hz, CH). ^{13}C NMR ($CDCl_3$, 100 MHz); δ_C 16.3 (CH_3), 17.6 (CH_3), 25.7 (CH_3), 26.5 (CH_2), 27.6 (C-5), 27.8 (C-7), 31.3 (C-4/9), 32.1 (C-1/3), 37.6 (C-8/10), 37.9 (C-6), 39.6 (CH_2), 46.5 (CH_2), 47.1 (CH_2), 49.5 (CH_2), 61.9 (C-2), 122.9 (CH), 124.1 (CH), 131.5 (C), 137.6 (C).

4.2.4. 8-Benzylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane (**11**) [13]

PCU-8,11-dione (Cookson's dione) was synthesized *via* photocyclization of Diels–Alder adducts obtained from reacting freshly cracked cyclopentadiene with *p*-benzoquinone [19,20]. PCU-dione (5.0 g, 28.7 mmol) was dissolved in anhydrous tetrahydrofuran (THF, 50 mL) and cooled with stirring to 5 °C with an external ice bath. Benzylamine (3.39 g, 31.6 mmol) was added slowly with continuous stirring while maintaining the temperature. The reaction mixture was stirred over 30 min and the resulting hydroxylamine (a white precipitate) was filtered and washed with cold THF. Dehydration of the hydroxylamine in dry benzene was achieved under Dean–Stark condition for 1 h or until no more water collected in the trap. The resulting solution was concentrated *in vacuo* to obtain the Schiff base (a yellow oil) which was reduced with $NaBH_4$ (1.63 g, 43.05 mmol) in dry methanol (30 mL) and dry THF (150 mL) with stirring for 24 h at room temperature. The solution was concentration *in vacuo* and water (2 × 100 mL) was added and the resulting mixture extracted with CH_2Cl_2 (4 × 50 mL) and the combined organic solution dried over $MgSO_4$ and concentrated *in vacuo* to yield a yellow oil. Purification was carried using column chromatography with solvent system; Hexane:CH_2Cl_2 (1:1) to obtain **11** as a colourless solid (Mp. 79–80 °C, 2.73 g, 36% yield, $R_f = 0.20$). ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.55 (AB, $J = 10.44$ Hz, 1H), 1.91 (AB, $J = 10.48$ Hz, 1H), 2.14 (br s, 1H, NH), 2.42 (t, $J = 4.9$ Hz, 1H), 2.51–2.62 (m, 4H), 2.71–2.84 (m, 3H), 3.98 (AB, $J = 13.36$ Hz, 1H), 4.03 (AB, $J = 13.36$ Hz, 1H), 4.66 (t, $J = 5.3$ Hz, 1H), 7.21–7.37 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 41.5 (CH), 42.0 (CH), 43.1 (CH), 43.2 (CH_2), 44.5 (CH), 44.8 (2 × CH), 47.8 (CH_2), 54.7 (CH), 55.2 (CH), 82.5 (CH), 109.5 (C), 126.8 (CH), 127.8 (CH), 128.3 (CH), 140.8 (C).

4.3. Synthesis of 8-amino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane (**12**)

To a solution of compound **11** (2.73 g, 10.3 mmol) dissolved in dry methanol (100 mL) was added 10% Pd/C (1.37 g) and stirred under hydrogen gas at atmospheric pressure for 16 h or until no starting material was observed on TLC. The spent Pd/C was filtered using celite and a sintered funnel, the solution was concentrated *in vacuo* and the crude product purified on silica using CH_2Cl_2:CH_3OH (95:5) to obtain **12** in pure form. A white solid (Mp. 143–145 °C, 1.66 g, yield 60.8% yield, $R_f = 0.52$). ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.50 (AB, $J = 10.44$ Hz, 1H), 1.86 (AB, $J = 10.48$ Hz, 1H), 2.09 (NH_2), 2.30–2.37 (m, 3H), 2.51–2.56 (m, 2H), 2.69–2.80 (m, 3H), 4.56 (t, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 41.4 (CH), 42.4 (CH), 43.3 (CH), 43.4 (CH_2), 44.8 (CH), 47.0 (CH), 55.1 (CH), 57.4 (CH), 83.0 (CH), 106.3 (C).

4.4. Synthesis of compound 8-chloroacetylamine-8,11-oxapentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]-undecane (**13**)

A mixture of compound **12** (1.62 g, 9.2 mmol), chloroacetyl chloride (0.807 mL, 10.1 mmol) and K_2CO_3 (2.54 g, 18.4 mmol) in dry DCM (20 mL) was stirred and heated gently at 40 °C for 1 h and allowed to stir overnight without heat. Purification was carried on silica, CH_2Cl_2:CH_3OH (95:5) to obtain pale yellow oil which solidified on standing at room temperature to afford a yellowish solid (Mp. 152–154 °C, 1.44 g, 62%, $R_f = 0.67$). ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.53 (AB, $J = 10.6$ Hz, 1H), 1.91 (AB, 10.6 Hz, 1H), 2.42 (s, 1H), 2.59–2.62 (m, 2H), 2.74–2.79 (m, 1H), 2.89–1.98 (m, 4H), 4.02 (s, 2H), 4.73 (t, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 41.4 (CH), 41.9 (CH), 42.6 (CH_2), 43.2 (CH_2), 43.6 (CH), 44.6 (CH), 44.8 (CH), 46.1 (CH), 55.0 (CH), 56.7 (CH), 83.7 (CH), 102.9 (C), 166.2 (C).

4.5. Synthesis of 8-[(E)-N-3,7-dimethylocta-2,6-dienylamino] acetamide-8,11-oxapentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]-undecane (**14**)

To a solution of geranylamine (0.33 g, 3.57 mmol) in dry THF (15 mL) was added K_2CO_3 (0.37 g) and compound **13** (0.45 g, 1.8 mmol), the mixture was stirred with reflux, and the reaction was monitored by TLC until no starting material was observed. The reaction mixture was cooled, filtered and concentrated *in vacuo*, the crude product was purified on silica gel; $CHCl_3$:CH_3OH (95:5) to obtain **14**. A yellow oil (380 mg, 57.5% yield, $R_f = 0.34$). IR ν_{max}: 3308, 2966, 1672, 1514, 1002 and 747 cm^{-1}. MS (TOF) calculated for $C_{23}H_{33}N_2O_2$ $(M + H^+)$ 369.2537, found 369.2537. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.54 (1H, AB, $J = 10.5$ Hz, H-4a), 1.57 (3H, s, CH_3), 1.58 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.90 (1H, AB, $J = 10.5$ Hz, H-4s), 1.95–2.06 (4H, m, 2 × CH_2), 2.41 (1H, t, $J = 4.76$ Hz, H-3), 2.53 (1H, m, H-5), 2.57–2.60 (1H, m, H-2), 2.69–2.73 (1H, m, H-6), 2.85–3.00 (4H, m, H-1, 7, 9, 10), 3.18 (2H, d, $J = 6.88$ Hz, CH_2), 3.19 (2H, s, CH_2), 4.69 (1H, t, $J = 5.16$ Hz, H-11), 5.02–5.06 (1H, m, CH), 5.13–5.17 (1H, m, CH), 7.94 (CONH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.7 (CH_3), 25.6 (CH_3), 26.4 (CH_2), 39.5 (CH_2), 41.4 (C-2), 41.8 (C-6), 43.4 (C-4), 43.5 (C-5), 44.6 (C-1), 44.8 (C-3), 45.9 (C-7), 47.1 (CH_2), 52.2 (CH_2), 54.8 (C-10), 56.4 (C-9), 83.5 (C-11), 102.2 (C-8), 121.7 (CH), 123.9 (CH), 131.6 (C), 139.0 (C), 172.1 (C═O).

4.6. Synthesis of 11-hydroxylpentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]-undecane-8-aminoethyl[(E)-N-3,7-dimethylocta-2,6-dien-amine] dihydrochloride (**15**)

Compound **14** (460 mg, 1.25 mmol) dissolved in dry THF (15 mL) was added LAH (0.24 g, 6.25 mmol) gently; the mixture was refluxed overnight under nitrogen atmosphere. The reaction vessel was cooled and the mixture quenched with aqueous Na_2SO_4, the obtained precipitate was filtered off and the filtrate concentrated to obtain the crude product. The crude product was purified *via* preparative HPLC (as specified above, retention time: 9.1 min) sample was lyophilized to obtain **15** (150 mg, 33.7% yield) and converted to its HCl salt to obtain a yellow slurry. IR ν_{max}: 3149, 3048, 2960, 2809, 1452, 1071 and 568 cm^{-1} MS (TOF) calculated for $C_{23}H_{37}N_2O$ ($M + H^+$ of free base) 357.2900, found 357.2897. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.08 (1H, AB, $J = 10.8$ Hz, H-4a), 1.53 (3H, s, CH_3), 1.61–1.63 (7H, m, H-4s, 2 × CH_3), 1.98–2.01 (4H, m, 2 × CH_2), 2.30–2.38 (3H, m, H-3, 5, 10), 2.50–2.55 (2H, m, H-2, 9), 2.64–2.69 (2H, m, H-1, 6), 2.80 (1H, s, H-7), 2.94 (s, 1H, H-8), 3.18 (4H, s, 2 × CH_2), 3.49 (2H, d, $J = 7.2$ Hz, CH_2), 3.86 (1H, t, $J = 3.2$ Hz, H-11),

4.98 (1H, t, J = 5.2 Hz, CH), 5.22 (1H, t, J = 6.7 Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.1 (CH_2), 34.0 (C-4), 34.9 (C-7), 37.8 (C-1), 39.5 (CH_2), 39.7 (C-2), 40.2 (C-6), 42.1 (C-9), 43.0 (C-3), 43.1 (CH_2), 43.2 (CH_2), 44.7 (C-5/10), 44.8 (C-5/10), 45.3 (CH_2), 57.4 (C-8), 70.4 (C-11), 114.2 (CH), 123.3 (CH), 131.9 (C), 145.2 (C).

4.7. Synthesis of 8,11-oxapentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]undecane-8-aminoethyl[(E)-N-3,7-dimethylocta-2,6-dien-amine] dihydrochloride (**16**)

To a solution of compound **14** (0.38 g, 1.03 mmol) in dry THF (10 mL) at 0 °C was added slowly 65% Red Al in toluene (1.02 mL, 5.16 mmol) and kept at this temperature for 10 min. The reaction was kept at 35 °C for an hour and then refluxed. The reaction was monitored until no starting material was observed on TLC. THF (20 mL) was added to the reaction mixture and quenched with 5 N NaOH (10 mL), the organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on silica gel; the column was first flushed with 100 mL of $CHCl_3$:CH_3OH (95:5) after which a $CHCl_3$:CH_3OH:NH_4OH (88:10:2) as eluent mixture was introduced to obtain **16** (165 mg, 45.2%, R_f = 0.57) in pure form and converted to its HCl salt to obtain a yellow slurry. IR ν_{max}: 3359, 2960, 1449, 1370, 1009 and 556 cm^{-1}. MS (TOF) calculated for $C_{23}H_{35}N_2O$ (M + H$^+$ of free base) 355.2744, found 355.2744. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.52 (1H, AB, J = 10.5 Hz, H-4a), 1.57 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.88 (1H, AB, J = 10.5 Hz, H-4s), 1.97–2.07 (4H, m, 2 × CH_2), 2.39 (1H, t, H-3), 2.46 (1H, t, J = 4.7 Hz, H-5), 2.50–2.59 (3H, m, H-2, 7, 9), 2.66–2.69 (1H, m, H-6), 2.73–2.81 (4H, m, H-1, 10, CH_2), 3.00 (2H, t, J = 5.4, 5.9 Hz, CH_2), 3.29 (2H, d, J = 6.96 Hz, CH_2), 4.58 (1H, t, J = 5.2 Hz, H-11), 5.03–5.07 (1H, m, CH), 5.22–5.26 (1H, m, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.5 (CH_2), 39.6 (CH_2), 41.5 (C-2), 41.9 (C-6), 42.3 (CH_2), 43.1 (C-5), 43.2 (C-4), 44.5 (C-1, 7), 44.8 (C-3), 46.3 (CH_2), 49.3 (CH_2), 54.7 (C-10), 55.1 (C-9), 82.5 (C-11), 110.1 (C-8), 120.2 (CH), 124.0 (CH), 131.8 (C), 140.4 (C).

4.8. Synthesis of pentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}] undecane-8,11-dione ethylene acetal (**17**) [28–30]

A mixture of PCU-dione **4** (10.0 g, 57 mmol), ethylene glycol (4.5 mL, 80 mmol) and *p*-toluenesulfonic acid (0.33 g, 1.9 mmol) was dissolved in toluene (45 mL) and refluxed using a Dean–Stark apparatus for three days. The reaction mixture was allowed to cool down followed by addition of cold aqueous solution of 10% (m/v) Na_2CO_3 (60 mL) and extracted with dichloromethane (3 × 25 mL). The organic layer was dried using anhydrous Na_2SO_4. The mixture was filtered and the solvent evaporated *in vacuo*. The crude product was purified *via* silica gel column chromatography (40:60; EtOAc: Hexane) and obtained as a white solid (Mp. 71 °C; 10.3 g, 80%, R_f = 0.55). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.55 (AB, J = 11.0 Hz, 1H), 1.84 (AB, J = 11.0 Hz, 1H), 2.37–2.38 (m, 1H), 2.44–2.47 (m, 1H), 2.51–2.63 (m, 3H), 2.74–2.79 (m, 2H), 2.89–2.94 (m, 1H), 3.80–3.91 (m, 4H). ^{13}C NMR [$CDCl_3$, 100 MHz] δ_C 36.3 (CH), 38.7 (CH_2), 41.3 (CH), 41.4 (CH), 42.2 (CH), 42.8 (CH), 45.8 (CH), 50.7 (CH), 52.9 (CH), 64.4 (CH_2), 65.6 (CH_2), 113.8 (C), 215.2 (C).

4.9. Synthesis of 4-benzyl-4-azahexacyclo[5.4.1.0.^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}] dodecan-3-ol (**19**)

A mixture of compound **17** (1.0 g, 4.59 mmol) and benzylamine (0.59 g, 5.5 mmol) in EtOH (10 mL) was heated at 100 °C using a sealed high pressure glass tube for 16 h. The solution was cooled and $NaBH_4$ (0.35 g, 9.17 mmol) was added gradually and the mixture was stirred at room temperature for 8 h. The solution was concentrated *in vacuo*, water (20 mL) was added and the intermediate was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic solution was washed with brine (15 mL) dried ($MgSO_4$) and concentrated *in vacuo*. To the crude product was added acetone (30 mL) and aqueous 4 M HCl (20 mL) with stirring at room temperature for 12 h. Water was added (250 mL) and the solution was basified to pH 14 with aqueous 1 M NaOH and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extract was dried over $MgSO_4$ and concentrated to obtain crude product, which was recrystallised from isopropanol to yield the desired product **19** (0.35 g, 29% yield, Mp. 158–159 °C), ^{1}H NMR [DMSO-d_6, 400 MHz]: δ_H 1.43 (AB, J = 10.2 Hz, 1H), 1.76 (AB, J = 10.2 Hz, 1H), 2.27 (t, J = 4.7 Hz, 1H), 2.46–2.51 (m, 4H), 2.60–2.69 (m, 2H), 2.77–2.80 (m, 1H), 3.17 (t, J = 5.0 Hz, 1H), 3.23 (br, 1H), 3.61 (d, J = 13.3 Hz, 1H), 5.76 (s, OH), 7.17–7.31 (m, 5H). ^{13}C NMR [DMSO-d_6, 100 MHz] δ_C 41.2 (CH), 41.5 (CH), 41.6 (CH), 41.8 (CH_2), 42.5 (CH), 44.7 (CH), 45.1 (CH), 50.4 (CH), 50.8 (CH_2), 55.3 (CH), 65.2 (CH), 105.9 (C), 126.2 (CH), 127.9 (CH), 128.4 (CH), 140.6 (C).

4.10. Synthesis of 4-azahexacyclo[5.4.1.0.^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (**20**)

To a solution of compound **18** (2.2 g, 8.3 mmol) in of dry CH_3OH (30 mL) was added gently 10% Pd/C (1.1 g) and stirred under H_2 at atmospheric pressure for 16 h or until no starting material was observed on TLC. The spent Pd/C was filtered using celite and a sintered funnel, the solution was concentrated *in vacuo* and the crude product purified on silica using $CHCl_3$:CH_3OH:NH_4OH (88:10:2) to obtain **19** in pure form as a white solid (Mp. 81–83 °C, 0.64 g, yield 44.1%, R_f = 0.30).^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.48 (AB, J = 10.5 Hz, 1H), 1.83 (AB, J = 10.4 Hz, 1H), 2.37–2.46 (m, 3H), 2.57–2.62 (m, 2H), 2.67–2.82 (m, 3H), 3.53 (t, J = 5.0 Hz, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 41.6 (CH), 41.8 (CH_2), 42.6 (CH), 43.4 (CH), 44.9 (CH), 45.8 (CH), 46.1 (CH), 54.5 (CH), 55.2 (CH), 60.5 (CH) 105.9 (C).

4.11. Synthesis of (E)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl) acetamide (**21**)

A mixture of chloroacetyl chloride (0.88 g, 7.8 mmol), geranylamine (1.0 g, 6.5 mmol) and K_2CO_3 (1.08 g, 7.8 mmol) in dry THF (30 mL) was refluxed for 16 h. The solution was filtered and concentrated *in vacuo* and purified on silica using $CHCl_3$:EtOAc (70:30) to afford (*E*)-2-chloro-*N*-(3,7-dimethylocta-2,6-dienyl) acetamide [**20**, 1.20 g, 81% yield, R_f = 0.80]. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.56 (s, 3H) 1.64 (s, 6H), 1.97–2.08 (m, 4H), 3.86 (t, J = 6.24 Hz, 2H), 4.00 (s, 2H), 5.03 (t, J = 6.72 Hz, 1H), 5.16 (t, J = 6.96 Hz, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.2 (CH_2), 37.7 (CH_2), 39.3 (CH_2), 42.5 (CH_2), 118.9 (CH), 123.6 (CH), 131.8 (C), 140.7 (C), 165.5 (C=O).

4.12. Synthesis of 4-[(E)-N-3,7-dimethylocta-2,6-dienyl]acetamide-4-azahexacyclo[5.4.1.0.^{2,6} .0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (**22**)

To a stirred solution of compound **20** (0.3 g, 1.7 mmol) in dry THF (15 mL) was added (*E*)-2-chloro-*N*-(3,7-dimethylocta-2,6-dienyl) acetamide (**21**, 0.39 g, 1.7 mmol) and K_2CO_3 (0.35 g, 2.55 mmol). The mixture was then refluxed overnight under a N_2 atmosphere. The reaction was allowed to cool to room temperature, filtered and concentrated *in vacuo*. The crude product was purified on silica using $CHCl_3$:CH_3OH (90:10) to afford the product **22** as light yellow oil (445 mg, 72% yield, R_f = 0.53). IR ν_{max}: 3230, 2966, 2868, 1661, 1562, 1348 and 728 cm^{-1}. MS (TOF) calculated for $C_{23}H_{33}N_2O_2$ (M + H$^+$) 369.2537, found 369.2539. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.49 (3H, s, CH_3), 1.52 (1H, m, H-12a), 1.56 (3H, s, CH_3), 1.57 (3H, s,

CH_3), 1.84 (1H, m, H-12s), 1.87–1.98 (4H, m, 2 × CH_2), 2.56 (1H, s, H-7), 2.66–2.70 (2H, m, H-8, 1), 2.78–2.84 (3H, m, H-11, 10, 2), 2.95 (1H, s, H-6), 3.06 (1H, s, H-9), 3.39 (br s, 1H), 3.71 (d, $J = 15.4$ Hz, 1H), 3.75 (2H, m, CH_2), 4.05 (1H, t, $J = 4.8$ Hz, H-5), 4.97 (1H, t, $J = 5.6$ Hz, CH), 5.08 (1H, t, $J = 6.3$ Hz, CH), 7.93 (s, CONH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.1 (CH_3), 17.5 (CH_3), 25.4 (CH_3), 26.2 (CH_2), 37.5 (CH_2), 39.3 (CH_2) 40.4 (C-9), 41.1 (C-12), 41.4 (C-8, 11), 43.2 (C-10), 43.8 (C-1), 46.2 (C-7), 47.5 (CH_2), 49.6 (C-6), 52.8 (C-2), 65.9 (C-5), 111.8 (C-3), 119.1 (CH), 123.6 (CH), 131.4 (C), 139.5 (C), 164.5 (C═O).

4.13. Synthesis of 4-[(E)-N-ethyl-3,7-dimethylocta-2,6-dienyl] amine-4-azahexacyclo[5.4.1.0 .^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol dihydrochloride (**23**)

To a solution of compound **22** (0.36 g, 0.97 mmol) in dry THF (10 mL) at 0 °C was added slowly 65% Red Al in toluene (0.58 mL, 2.9 mmol) and the mixture was kept at this temperature for 10 min. The reaction was kept at 35 °C for an hour and refluxed; the reaction was monitored until no starting material was observed on LC-MS. THF (20 mL) was added to the reaction mixture and quenched with 5 N NaOH (10 mL), the organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was dissolved in methanol (5 mL) and purified using preparative HPLC (as specified above, retention time: 13.6 min). The sample was lyophilized to obtain **23** (200 mg, 55% yield) and converted to its HCl salt to obtain a light yellow slurry. IR ν_{max}: 2967, 2866, 1668, 1198, 1178, 1129, 831 and 719 cm^{-1}. MS (TOF) calculated for $C_{23}H_{35}N_2O$ ($M + H^+$ of free base) 355.2744, found 355.2748. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.57 (3H, s, CH_3), 1.60 (1H, H-12a), 1.65 (6H, s, 2 × CH_3), 1.93 (1H, d, $J = 10.92$ Hz, H-12s), 2.00–2.07 (4H, m, 2 × CH_2), 2.60 (1H, m, H-7), 2.73 (1H, m, H-8), 2.78 (1H, m, H-1), 2.83–2.87 (3H, m, H-11, 10, 2), 2.96–2.98 (1H, m, H-6), 3.01–3.24 (5H, m, H-9, 2 × CH_2), 3.41 (2H, d, $J = 7.16$ Hz, CH_2), 3.90 (1H, t, $J = 5.1$ Hz, H-5), 5.03 (1H, m, CH), 5.21 (1H, t, $J = 6.5$ Hz, CH), 5.61 (OH, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.3 (CH_2), 39.6 (CH_2), 41.1 (C-9), 41.2 (C-12), 41.6 (C-8), 41.7 (C-11), 43.5 (C-10), 44.1 (C-1), 44.3 (CH_2), 44.5 (CH_2), 45.9 (CH_2), 46.3 (C-7), 50.1 (C-6), 53.5 (C-2), 67.3 (C-5), 113.5 (C-3), 117.0 (CH), 123.7 (CH), 132.1 (C), 143.4 (C).

4.14. Biological testing

Compounds **9**, **10**, **14**, **15**, **16**, **22**, **23** and SQ109 were first dissolved in 100% Methanol, sonicated and filter sterilized using 0.22 μm polycarbonate sterile filters to obtain a stock concentration of 10 mg/mL. This was diluted in sterile water and twofold serial dilutions were made to give working concentration ranges of 8 μg/mL to 0.125 μg/mL. A 1 mL volume of each concentration was aliquoted into cryovials and stored at −70 °C.

4.15. Bactec MGIT 960 analysis

Susceptibility testing with the BACTEC MGIT 960 system (Becton Dickinson) was performed according to the manufacturer's recommendations. Mycobacterial work was carried out in a level III biosafety laboratory. *M. tuberculosis* reference strain H_{37}Rv (ATCC No. 25177), MDR (drug sensitivity: isoniazid > 0.2 μg/mL, rifampicin > 1.0 μg/mL and EMB > 5 μg/mL) and XDR strains (drug sensitivity: isoniazid > 0.2 μg/mL, rifampicin > 1.0 μg/mL, EMB > 5 μg/mL, streptomycin > 2.0 μg/mL, ofloxacin > 2.0 μg/mL and kanamycin > 5.0 μg/mL) were cultured in Middlebrook 7H9 medium [31], enriched with OADC (0.00 5%, v/v, oleic acid; 0.5%, 171 w/v, BSA; 0.2%, w/v, glucose; 0.02%, v/v, catalase and 0.085%, w/v, NaCl) and incubated at 37 °C. Freshly grown cultures were used to prepare a standardised inoculum in a sterile tube containing 4.5 mL phosphate buffer, 0.05% tween 80 with glass beads (5 mm diameter) by vortexing. Once the clumps were allowed to settle for 45 min, the supernatant was aspirated and adjusted to a McFarland No. 1 standard, equivalent to a 10^7 colony forming units CFU/mL. 0.5 mL of the standardised inoculum was diluted tenfold to obtain a final concentration of 10^5 CFU/mL, after which 0.5 mL of the standardised inoculum was added to each of the MGIT containing the compounds. A 1:100 inoculum dilution was used to inoculate the drug free control tubes. This represents 1% of the bacterial population. The MGITs were loaded in the BACTEC 960 drawers and the MIC was determined to be the lowest dilutions that were negative by the automated system in the compound containing tubes when the control tube showed positive. Antimycobacterial analysis of all compounds was done in triplicate.

All compounds containing tubes belonging to the positive drug free control were unloaded from the BACTEC 960 system and a Ziehl Neelsen stain was performed to confirm the presence of *M. tuberculosis* [32]. Colony counts of the test inoculum were also prepared by plating out 20 μL onto Middlebrook 7H11 agar plates. Plates were incubated aerobically at 37 °C for 21 days. SQ109 was used as the reference drug.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.03.041.

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