European Journal of Medicinal Chemistry 44 (2009) 4297–4305

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Pentacyclo-undecane derived cyclic tetra-amines: Synthesis and evaluation as potent anti-tuberculosis agents

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ARTICLE INFO

Article history:
Received 11 March 2009
Received in revised form
15 July 2009
Accepted 16 July 2009
Available online 22 July 2009

Keywords:
XDR-tuberculosis (TB)
Pentacycloundecane
Isoprenyl
SQ109

ABSTRACT

As part of an ongoing effort to develop highly potent anti-tuberculosis agents, fourteen pentacyclo-undecane (PCU) tetra-amine compounds were synthesized and screened for their in vitro anti-*mycobacterial* activity against two TB strains, H37Rv and XDR 194 [an extensively drug-resistant strain of tuberculosis]. Using the broth macrodilution method, nitrofuranylamide based compounds (**6a** and **6b**) showed almost similar activities against the H37Rv strain of *Mycobacterium tuberculosis* when compared with the control drug, ethambutol. *N*-Geranyl piperazine PCU (**8a**) and *trans–trans* farnesyl piperazine PCU (**8b**) were 3.2 and 3.7 times more potent than commercially available ethambutol. Both isoprenyl PCU tetra-amine derivatives and *N*-decyl piperazine PCU (**9a**) were highly active against the XDR 194 strain of tuberculosis with MICs in the range of 0.63–3.02 μM. Cytotoxicities (IC_{50}) of isoprenyl based compounds (**8a**, **8b**) and compound **9a** were tested on a mammalian cell line [MDBK (Madin Darby bovine kidney epithelium)] with values of 30, 24 and 25 μM respectively.

1. Introduction

The WHO estimate of 9.2 million new tuberculosis cases with 1.7 million deaths in 2006, indicates that tuberculosis (TB) still accounts for a significantly large proportion of the global disease burden and mortality [1]. India, China, Indonesia, South Africa and Nigeria featured as the top five of the highest affected countries. The highest incidence rates were found in Africa, with twelve of the fifteen countries in this continent having the most severe infection rates.

The worldwide increase in prevalence of *Mycobacterium. tuberculosis* and the emergence of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB has imposed a serious setback on global TB control [2]. There were approximately 0.5 million reported cases of MDR-TB throughout the world in 2006 [2]. Aside from protracted treatment periods [2] and the use of more toxic and expensive second line drugs [3], MDR-TB is associated with low cure rates and high mortality [4]. XDR-TB found in most parts of the world [3] is almost untreatable and is accompanied by higher mortality rates than MDR-TB [5].

The difficulties associated with the long duration of therapy, such as patient non-compliance following improvement after the intensive phase and ingestion of multiple doses consisting of many tablets, have also led to a need to develop simple drug regimen. The ensuing limited treatment options for MDR and XDR-TB have created a renewed interest in the development of novel anti-TB drug candidates. These new drugs must effectively shorten treatment time and act against the subpopulation of slowly metabolising *bacilli*.

Among the promising potential anti-TB drugs currently undergoing human clinical trials are the third generation fluoroquinolones, gatifloxacin and moxifloxacin, diarylquinolone TMC 207, nitroimidazole PA-824 and nitroimidazo-oxazole OPC-67683 [6]. Other drugs that are in the preclinical phase include the diamine SQ109 (2), dipiperidines SQ609, nitroimidazo-oxazole back-up, synthase inhibitor FAS20013, translocase I inhibitors and non-fluorinated quinolones [6].

SQ109 (*N*-geranyl-*N*′-(2-adamantyl)ethane-1,2-diamine), developed by Sequella Inc. was first synthesized by Lee et al.[7] using solid phase synthesis of 1,2 diamine analogues of ethambutol (EMB) (**1**) (Fig. 1). From the several active 1,2 diamine derivatives

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doi:10.1016/j.ejmech.2009.07.015

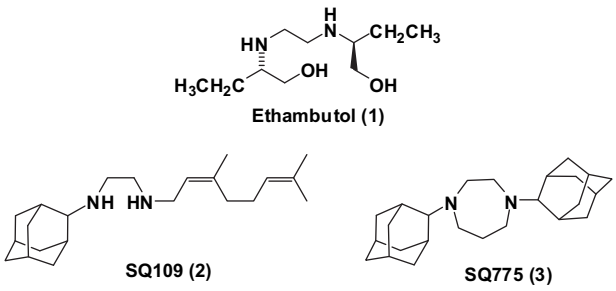


Fig. 1. Structures of ethambutol, SQ109 and SQ 775.

obtained using a combinatorial approach, SQ109 (**2**) was found to be the most potent new anti-TB lead. SQ109 (**2**) is an effective compound against EMB resistant strains [8]. Its activity against XDR-TB strains and a 25% reduction in the time to cure mice by enhancing the activity of isoniazid and rifampicin was also reported. SQ 775 (**3**) is an example of a cyclic diamine compound with an activity similar to the control drug EMB [9].

Non-TB related research showed that polycyclic "cage" compounds (such as adamantane and pentacyclo-undecane) can improve drug lipophilicity, thus serving as a transport aid in carrying such drugs across the Blood Brain Barrier (BBB) or Central Nervous System (CNS) [10,11]. It can also reduce the bio-degradation of the drug thereby prolonging the pharmaceutical effect of such agents in the body [11–14]. SQ109 and SQ775 both have an adamantane moiety in common suggesting that this skeleton might be contributing to its lipophilicity thereby enhancing their anti-TB activities.

In a similar development, Tangallapally et al. [15,16] recently reported novel nitrofuranylamide compounds with promising in vitro anti-tuberculosis activities. However, this activity could not be duplicated in *in vivo* analysis due to a short serum half life and rapid elimination of the compound. This led to the introduction of a bicyclic (tetrahydroisoquinoline) moiety in order to make it more resistant to proteolysis [17]. This particular modification showed an improvement in serum half life while improving its MIC activity (Fig. 2) for compound **4** [17].

For our work it was proposed that the replacement of the bicyclic tetrahydroisoquinoline with a more rigid lipophilic compound such as the pentacycloundecane (PCU) moiety, a polycyclic "cage", might further enhance the activity by facilitating the movement of these molecules across the lipid-enriched bacterial cell membrane.

Inspired by the work of Lee et al. [7] and Tangallapally et al. [15–17] a series of novel amine based compounds (**5**–**13**) bearing the lipophilic PCU molecule were designed and synthesized (Fig. 3).

2. Chemistry

The starting material, PCU ditosylate [18] was synthesized from pentacyclo-undecane-8,11-dione as illustrated in Scheme 1. The starting material, Cookson's dione (**14**) [19] was reacted with freshly prepared allyl magnesium bromide (Grignard reaction) to afford the *endo*-8,*endo*-11 diol (**15**) which upon dehydration under Dean–Stark conditions, yielded the corresponding 3,5-dially-4-oxahexacyclo [5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$] dodecane (**16**) [20]. Ozonolysis of the hexacyclic ether (**16**) followed by reductive work up yielded the PCU diol (**17**) [18] which upon tosylation gave the PCU ditosylate (**18**) [18]. The overall yield of **18** from **14** was 60%.

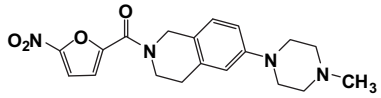
PCU ditosylate (**18**) was reacted with excess piperazine/homopiperazine in dry dichloromethane at −78 °C to afford the tetra-amines **5a** and **5b** respectively (62%) and these were reacted with 5-nitrofuran-2-carbonyl chloride (**19**) to obtain compounds **6a** and **6b** respectively (65%). PCU ditosylate (**18**) was reacted with *N*-benzyl homopiperazine (**20**) at a 1:2.2 ratio with triethylamine under reflux conditions to obtain compound **7** (Scheme 2).

Compound **5a** was successfully reacted with geranyl bromide (**21a**) to afford compound **8a** but its purification proved difficult *via* column chromatography. To solve this problem, a new synthetic route was introduced. Applying the methodology employed to synthesize compound **5a**, geranyl bromide (**21a**) was reacted with excess piperazine/homopiperazine to obtain *N*-geranyl piperazine (**22a**) and *N*-geranyl homopiperazine (**23a**) while the reaction of *trans–trans* farnesyl bromide (**21b**) with piperazine/homopiperazine afforded compounds **22b** and **23b**. Piperazine was also reacted with three linear alkane chains (C10, C15 and C20) to obtain compounds **25a**, **25b** and **25c** (Scheme 3).

Reaction of PCU ditosylate (**18**) with compound **22a** and **22b** in the presence of K_2CO_3 under reflux resulted in compounds **8a** and **8b** respectively (yield 71%) which were easily purified *via* column chromatography. Attempts to react PCU ditosylate (**19**) with **23a** and **23b** were unsuccessful. PCU ditosylate (**18**) was reacted with **25a**, **25b** and **25c** to yield linear alkane derivatives of PCU dipiperazine **9a**, **9b** and **9c** respectively. Reaction of the PCU ditosylate (**18**) with *N*-benzoyl piperazine (**26**) yielded compound **10**, followed by the reduction of the carbonyl group using lithium aluminium hydride to obtain the corresponding benzyl tetra-amine **11** in 55% yield (Scheme 4).

2-(Aminomethyl) pyridine (**27**) and ethanolamine (**28**) were reacted with benzaldehyde (**29**) *via* reductive amination to obtain their corresponding secondary amines, *N*-benzyl-*N*-{(pyridin-2-yl)methyl}amine (**30**) [21,22] and 2-(benzylamino)ethanol (**31**) [23] (Scheme 5).

N-Benzyl-*N*-{(pyridin-2-yl)methyl}amine (**30**) was reacted with PCU ditosylate (**18**) to obtain compound **32** after which the benzyl protecting groups were removed by catalytic hydrogenation using ammonium formate with 10% palladium on carbon to obtain compound **12** (yield 67%). 2-(Benzylamino) ethanol (**31**) was treated with PCU ditosylate (**18**) to obtain compound **13** in an overall yield from **18** of 70% (Scheme 6).



4

Fig. 2. Lead compound **4**.

3. Results and discussion

All synthesized novel PCU amine derivatives **5**–**13** excluding **9** were screened in vitro against *M. tuberculosis* H37Rv (ATCC 25618) using a broth macrodilution method (BMM). The *C*log *P* of each novel compound was obtained using the ACDLABS LogP 11.0 program.[1] The results are depicted in Table 1.

MIC results were obtained after 21 days of incubation. Compounds **5a** and **5b** differ in structure only by the ring sizes of the diamines. Compound **5b** shows low activity against the H37Rv strain, while **5a** did not show any activity at the highest concentration tested. Modification to these compounds with the introduction of a nitrofuran group to form compounds **6a** and **6b** gave promising activity with MIC values of 24.1 and 23.1 μM respectively,

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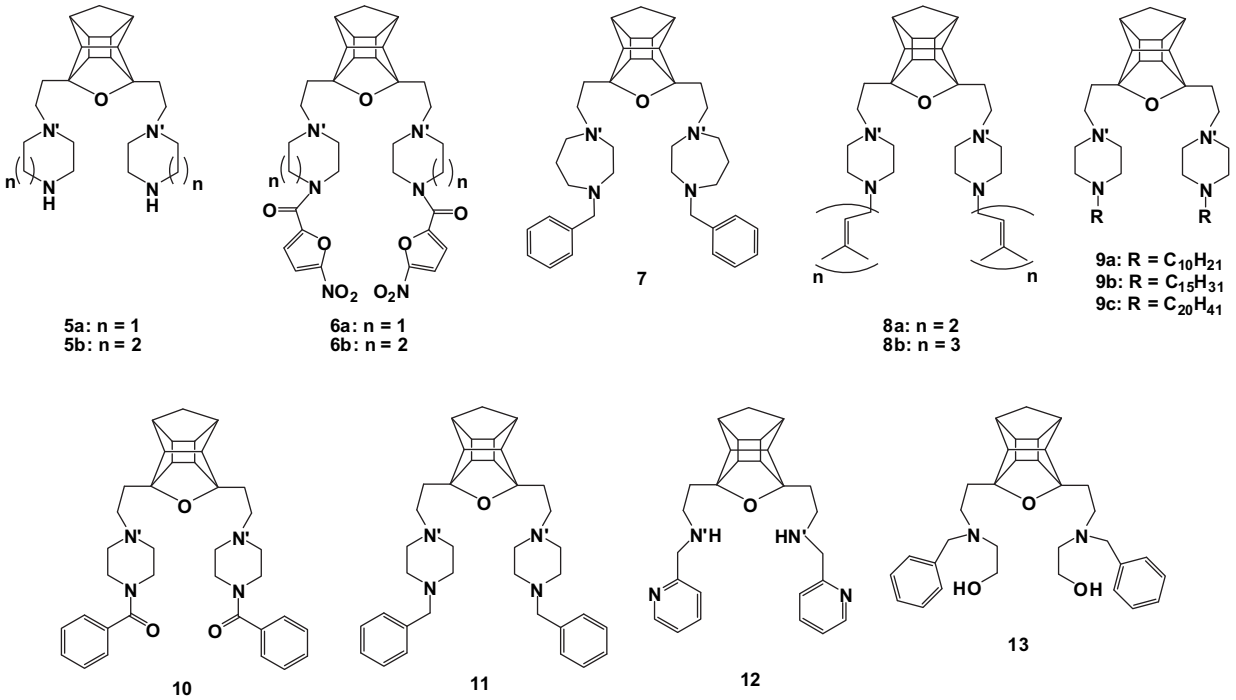
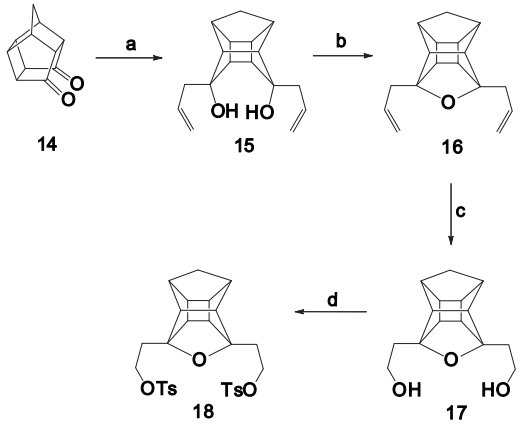


Fig. 3. PCU amine derivatives **5**–**13**.

which are similar to the control drug, EMB. Compounds **6a** and **6b** did not show any significant difference in calculated *C*log *P* values when compared to **5a** and **5b**.

Compound **7** (the benzyl derivative of **5b**) exhibits similar activity to **5b** (μg/mL) while compound **11** (the benzyl derivative of **5a**) demonstrates an improved activity against H37Rv, whilst **5a** did not show comparable activity. The benzoyl derivative **10** (of **5a**) showed a weaker potency compared to **11**. Compounds **12** and **13** did not show any significant activity.

Based on results obtained by Lee et al. [7,8] in the development of SQ109 (**2**), it was decided to synthesize the geranyl and *trans–trans* farnesyl derivative of **5a** and **5b**. As discussed above, the isoprenyl derivatives of **5b** proved to be elusive. Compounds **8a** and **8b** had MIC values of 6.09 and 5.04 μM respectively. The activity of these lipophilic alkene bearing compounds (**8a** and **8b**) were similar (expressed in μg/mL) to EMB, when screened against H37Rv.

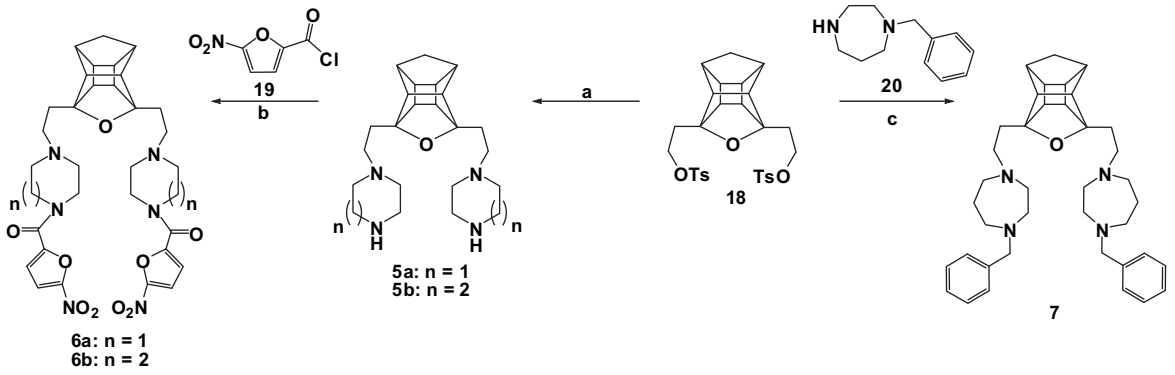


Scheme 1. Reagents and conditions: (a) $H_2C{=}CHCH_2MgBr$, dry THF; (b) Dean–Stark apparatus, H_2SO_4, benzene, reflux; (c) O_3, dry CH_3OH, $NaBH_4$; (d) *p*-TsCl, powdered KOH, THF.

Based on the results for compounds **6a**, **6b**, **8a** and **8b** we decided to test the molecules with a more accurate method (BACTEC 460 TB system) against the H37Rv strain. Compounds **6a** and **6b** (Table 2) showed little difference when compared to the results obtained from the BMM method. Further screening was not carried out, although it is known that MICs of drugs differ slightly depending on the assay method used [25]. Due to the activity of **8a** and **8b** we decided to investigate the effect of using long alkane chains in place of the alkene derivatives to determine whether this functionality is essential for efficacy. This led to the synthesis of compounds **9a** (ten carbon alkane), **9b** (fifteen carbon alkane) and **9c** (twenty carbon alkane) (Scheme 4). Anti-mycobacterial screening of compounds **9b** and **9c** was not possible however; due to their insolubility at biological pH presumably due to their highly lipophilic nature (*C*log *P* values are 14.43 ± 0.52 and 19.74 ± 0.52 respectively). Compounds **8a**, **8b** and **9a** were tested against an XDR strain of *M. tuberculosis* [strain X194, resistant to first line drugs (Isoniazid and Rifampicin) and second line drugs (Kanamycin, Ofloxacin and Amikacin)] (BACTEC 460 TB system). These compounds were further analyzed for cytotoxicity on mammalian cells from the MDBK (Madin Darby bovine kidney epithelium) line (Table 2).

Compounds **8a** and **8b** show potent activity against the H37Rv strain with **8b** being approximately two-fold more active than **8a** against both H37Rv and XDR 194 strains respectively. The MIC for EMB on H37Rv is 0.94 μg/mL [25] the MIC for **8b** against H37Rv was lower (0.5–0.25 μg/mL, Table 2). The XDR strain used was resistant to EMB at the breakpoint concentration of 2.5 μg/mL in BACTEC [24]. Therefore, the MIC for the XDR strain lies beyond 2.5 μg/mL. This means that the efficacy of compound **8b** is between 2.5 and 5 times more effective in inhibiting the XDR strain than EMB (**8b** killed the strain between 1 μg/mL and 0.5 μg/mL, Table 2). Also, the sensitivity for **8b** between H37Rv and X194 was approximately two fold (Table 2). The results indicate that our compounds probably have a different cellular target to that of EMB.

9a proved to be twice as active as compound **8a** against the XDR strain of *M. tuberculosis*. It was not considered necessary to test **9a**

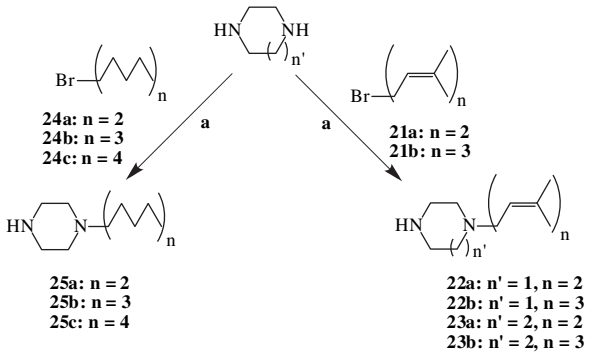


Scheme 2. Reagents and conditions: (a) piperazine/homopiperazine, DCM, −78 °C, rt, 24 hrs; (b) DCM, Et_3N, reflux 16 h; (c) CH_3CN, Et_3N, reflux, N_2 atm.

using the BMM method since the BACTEC system is much more accurate. Compound **9a** has a higher *C*log *P* value compared to **8a** (Table 2), suggesting that lipophilicity could play an important role in the activity of this class of compounds. However, this assumption could not be further evaluated due to insolubility problems encountered with compounds **9b** and **9c**. It should be noted that the MICs for **9a** and **8b** (in ug/ml) fall in the same range ($1 > MIC > 0.5$), however conversion to μM makes quite a significant difference as compound **8b** has a higher molar activity than **9a** (**8b** has a larger molecular weight).

Compounds **8a** and **8b** are structurally related to SQ109, they all have polycyclic "cage" moieties, diamines (linear or cyclic) and long alkene chains, which form the basis of this comparison. As illustrated in Table 2, **8a** is only half as active as SQ109 whereas **8b** is twice as active as SQ109 [8]. This suggests that the length of the alkene chain (lipophilicity again) may be playing an important role in the activity of these compounds. The cytotoxicities (IC_{50}, Table 2) of these compounds (**8a**, **8b** and **9a**) and that of SQ109 fall in the same range between 20 and 30 μM with compound **8a** being the least toxic.

A potential anti-TB candidate needs to be evaluated as a drug combination, i.e. the possibility of incorporating such an anti-TB candidate with other existing anti-TB drugs. Based on this, further studies were carried out to investigate the interaction of compound **8b** (the most active compound in this series) with two known anti-TB drugs (Isoniazid and Rifampicin) by means of in vitro testing against the H37Rv strain of *M. tuberculosis*. The interaction of compound **8b** with these two anti-TB drugs could be antagonist, additive, synergistic or have no effect at all. Results obtained using once again the BACTEC 460 TB system showed that compound **8b** (0.5 μg/mL) had no antagonist or synergistic effect; it did however have an additive effect.



Scheme 3. Reagents and conditions: (a) piperazine/homopiperazine, DCM, −78 °C, rt, 24 h.

4. Conclusion

A series of novel PCU tetra-amines (fourteen compounds) were synthesized and screened for their anti-TB activity of which five compounds (**6a**, **6b**, **8a**, **8b** and **9a**) displayed the highest anti-TB activity against *M. tuberculosis* H37Rv. Compounds **8a**, **8b** and **9a** were the most potent with excellent activities giving MICs of 3.04, 1.26 and 1.50 μM respectively against an XDR-TB strain (X194). These results indicate that lipophilicity is an important component of their efficacy (having larger *C*log *P* values than SQ109). This does however limit their potential application due to solubility problems (as seen for molecules **9b** and **9c**). We are examining the design of new derivatives in terms of their structure–activity relationship. It is hoped that new more soluble active species can be obtained. The first generation of these active compounds however, is currently undergoing further *in vitro* and *in vivo* analysis.

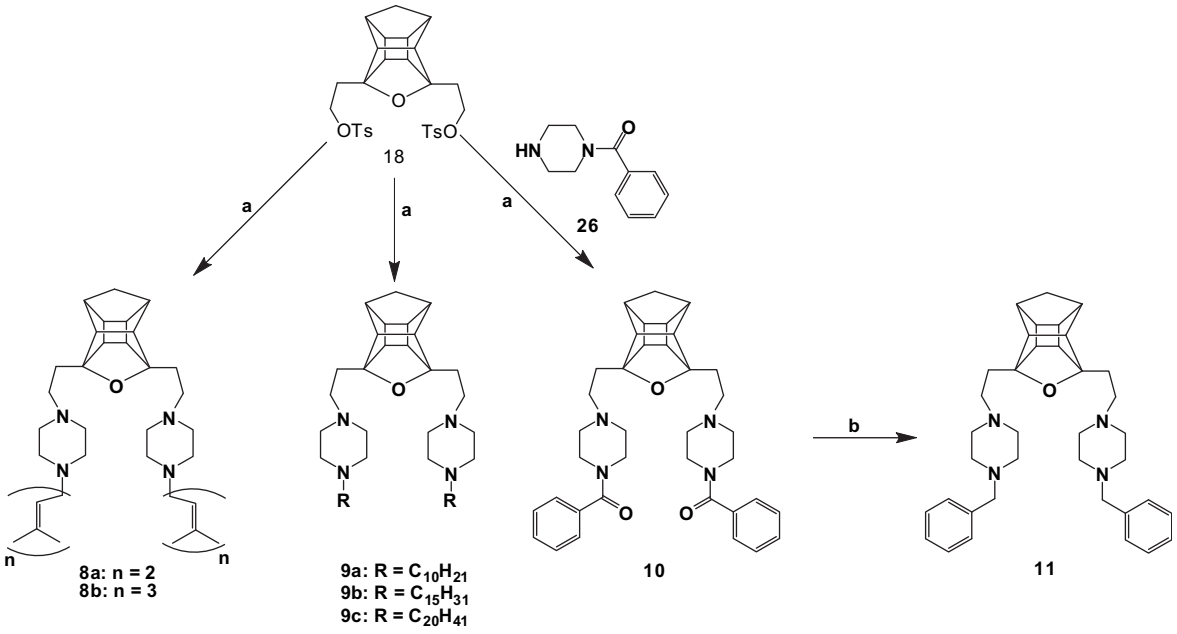
5. Experimental

The NMR 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 pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 instrument with an Attenuated Total Reflectance attachment recorded in cm^{-1}. All reactions were monitored using Thin Layer Chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by Column Chromatography using Fluka Kieselgel 60 (70–230 mesh) and CH_3Cl:CH_3OH:NH_4OH (88:10:2) as the eluent (solvent mixture). Level of purity for all compounds was judged to be >95% based upon ^1H NMR and LC-MS analysis. Mass Spectra were obtained using a Waters LCT Premier Time of Flight mass spectrometer. Tetrahydrofuran was freshly distilled before use from a sodium benzophenone under N_2 atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use. The syntheses of the precursors are described in their corresponding references.

*C*log *P* gives an indication of the lipophilicity of the drug with reference to its pharmacological importance (pharmacokinetics and pharmacodynamics) [15,26]. The values were calculated using ACD/Labs LogP software v11.0.

5.1. Synthesis of PCU tetra-amine *5a* & *5b*

To a vigorously stirred solution of the cyclic diamines (piperazine/homopiperazine) (22 mmol) in DCM (400 mL) at −78 °C (dry ice, 2-propanol) under N_2 atmosphere was added dropwise PCU ditosylate



Scheme 4. Reagents and conditions: (a) **22** or **25** or **26**, K_2CO_3, CH_3CN, reflux under N_2 atm; (b) $LiAlH_4$, dry THF and reflux under N_2 atm, 36 h.

(**18**, 1.2 g, 2.2 mmol) in DCM (100 mL) over 45 min. The reaction mixture was left to attain room temperature with stirring for 24 h. The solution was washed with water to remove excess diamines, the obtained organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified *via* column chromatography using CH_3Cl:MeOH:NH_4OH (88:10:2) to obtain a pure product.

*5.1.1. PCU dipiperazine (**5a**)*

A yellow oil ($R_f = 0.2$, 0.56 g, 68% yield). IR ν_{max}: broad absorption (N–H) 3367, 2949, 1655, 1463, 1125 and 746 cm^{-1}. HRMS calculated for $C_{23}H_{36}N_4O$ ($M + H^+$) 385.2955, found 385.2967. ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.52 (*AB*, $J_{AB} = 10.1$ Hz, 1H), 1.87 (*AB*, $J_{AB} = 10.1$ Hz, 1H), 1.99 (t, $J = 7.92$ Hz, 2H), 2.38–2.60 (m, 8H), 2.95 (2H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.7 (t), 41.8 (d), 43.4 (t), 44.5 (d), 45.6 (t), 48.0 (d), 53.7 (t), 55.1 (t), 58.8 (d), 94.87 (s).

*5.1.2. PCU dihomopiperazine (**5b**)*

A light yellow oil, ($R_f = 0.2$, 0.55 g, 62%). IR ν_{max}: broad absorption (N–H) 3386, 2818, 1465, 1108, 927, 918 and 748 cm^{-1}. HRMS calculated for $C_{25}H_{40}N_4O$ ($M + H^+$) 413.3280 found 413.3287. ^1H NMR [$CDCl_3$, 400 MHz] δ_H 1.48 (*AB*, $J_{AB} = 10.2$ Hz, 1H), 1.84 (*AB*, $J_{AB} = 10.2$ Hz, 1H), 1.76 (2H), 1.98 (2H), 2.35–2.69 (m, 10H), 2.89–2.94 (m, 4H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 30.0 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.5 (d), 47.2 (t), 48.0 (d), 48.6 (d), 54.3–55.0 (t), 57.8 (t), 58.8 (d), 94.8 (s).

*5.2. Synthesis of 5-nitrofuran-2-carbonyl diamine PCU **6a** & **6b***

To a stirred mixture of PCU tetra-amines (**5a** and **5b**, 1.2 mmol) in DCM (2 mL) and Et_3N (670 μL, 4.8 mmol) under N_2 atmosphere was added freshly prepared 5-nitrofuran-2-carbonyl chloride (**19** [15] 3.6 mmol) in DCM (3 mL) and stirred for 18 h at reflux. The reaction mixture was cooled and diluted with 60 mL of ethyl acetate and washed sequentially with (2×50 mL) 10% $NaHCO_3$, (2×50 mL) water and (2×50 mL) brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified *via* column chromatography using CH_3Cl:MeOH:NH_4OH (88:10:2).

*5.2.1. 5-Nitrofuran-2-carbonyl piperazine PCU (**6a**)*

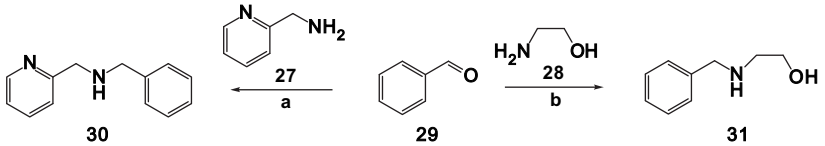
A brown oil ($R_f = 0.7$, 0.56 g, 65%). IR ν_{max}: 2927, 1630, 1352, 1021 and 752 cm^{-1}. HRMS calculated for $C_{33}H_{38}N_6O_9$ ($M + H^+$) 663.2779, found 663.2758. ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.52 ($J_{AB} = 10.1$ Hz, 1H), 1.87 ($J_{AB} = 10.1$ Hz, 1H), 1.99 (t, $J = 7.36$ Hz, 2H), 2.38–2.61 (m, 10H), 3.76 (s, 2H), 3.85 (s, 2H), 7.15 (1H), 7.32 (1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.9 (t), 41.8 (d), 43.1 (t), 43.1 (t), 43.5 (t), 44.5 (d), 46.6 (t), 48.1 (d), 53.4 (t), 54.4 (t), 58.8 (d), 94.8 (s), 111.7 (d), 118.1 (d), 148.7 (s), 151.2 (s), 156.6 (s).

*5.2.2. 5-Nitrofuran-2-carbonyl homopiperazine PCU (**6b**)*

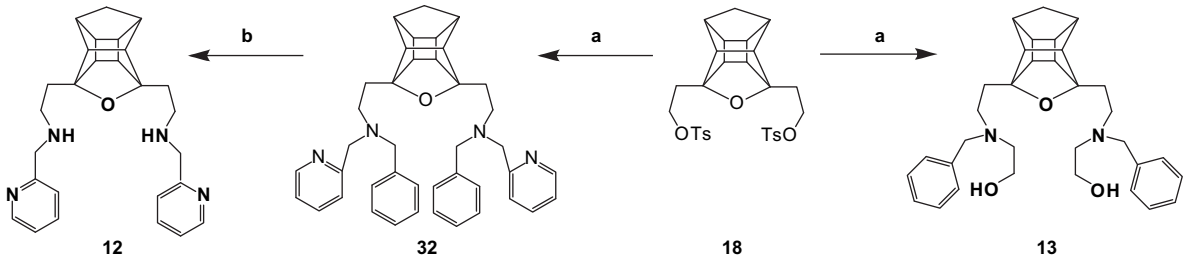
A brown oil ($R_f = 0.51$ g, 61%). IR ν_{max}: 3479, 2951, 2859, 1627, 1530, 1352, 810 and 729 cm^{-1}. HRMS calculated for $C_{35}H_{42}N_6O_9$ ($M + H^+$) 691.3092, found 691.3105. ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.52 ($J_{AB} = 10.1$ Hz, 1H), 1.87 ($J_{AB} = 10.1$ Hz, 1H), 1.96–2.03 (m, 4H) 2.37–2.88 (m, 10H), 3.72–3.77 (m, 2H), 3.84 (2H), 7.19 (1H), 7.34 (1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 27.0 (t), 28.7 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.4 (d), 46.2 (t), 47.3 (t), 47.9 (t), 48.0 (d), 48.8 (t), 54.2–54.9 (t), 54.7 (t), 58.8 (d), 94.8 (s), 111.7 (d), 118.0 (d), 149.2 (s), 151.2 (s), 157.9 (s).

5.3. Synthesis of N*-benzyl homopiperazine PCU (**7**)*

A mixture of *N*-benzyl homopiperazine (**20**, 0.7 g, 3.68 mmol) and PCU ditosylate (**18**, 0.93 g, 1.67 mmol) and triethylamine (350 μL, 2.5 mmol) in CH_3CN (20 mL) was refluxed for four days



Scheme 5. Reagents and conditions: (a) CH_3CH_2OH, rt, l h, $NaBH_4$, reflux, stir overnight, N_2 atm; (b) CH_3OH, rt, 2 h, $NaBH_4$, stir overnight, N_2 atm.



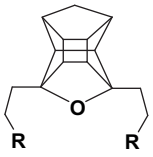
Scheme 6. Reagents and conditions: (a) **30** or **31**, CH_3CN, Et_3N, reflux, N_2 atm; (b) 10% Pd/C, ammonium formate, dry MeOH, reflux, 16 h, N_2 atm.

under N_2 atmosphere. The reaction mixture was cooled, filtered and concentrated *in vacuo*. The crude residue was purified *via* column chromatography on silica gel using CH_3Cl:MeOH:NH_4OH (88:10:2, $R_f = 0.65$) as eluent to give the product as a yellow oil (0.84 g, 85%). IR ν_{max}: 2935, 2811, 1452, 1351, 1111, 729 and 695 cm^{-1}. HRMS calculated for $C_{39}H_{52}N_4O$ ($M + H^+$) 593.4219 found 593.4230. ^1H NMR [$CDCl_3$, 400 MHz] δ_H 1.48 ($J_{AB} = 10.2$ Hz, 1H), 1.84 ($J_{AB} = 10.2$ Hz, 1H), 1.77 (2H), 1.96 (t, $J = 8.0$ Hz, 2H), 2.35–2.73 (m, 14H), 3.60 (s, 2H), 7.26–7.31 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 27.5 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.4–55.1 (t), 58.8 (d) 62.8 (t), 94.9 (s), 126.8 (d), 128.2 (d), 128.9 (d), 139.5 (s).

5.4. *Synthesis of* N-*isoprenyl/linear alkane diamines*

To a vigorously stirred solution of diamine (piperazine/homopiperazine) (10 mmol) in DCM (750 mL) at −78 °C (dry ice, 2-propanol) under N_2 atmosphere was added dropwise a solution of isoprenyl bromide (**21a** or **21b**, 2 mmol)/linear alkane bromide (**24a**, **24b** or **24c**, 2 mmol) in DCM (250 mL) over 45 min. The reaction mixture was left to attain room temperature with stirring for 24 h. The solution was washed with water to remove excess piperazine, the organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified *via* column chromatography using CH_3Cl:MeOH:NH_4OH (88:10:2).

Table 1
Clog P values and in vitro anti-mycobacterial BMM activity on day 21 of novel PCU amine compounds against *M. tuberculosis* H37Rv.



Compound	R	MW	*Clog P*[a]	H37Rv (MIC)	
				μg/mL	μM
1	Ethambutol	204	NA	4	19.6
5a	$-N'(CH_2)_4NH$	385	0.41 ± 0.55	>128	ND
5b	$-N'(CH_2)_5NH$	413	1.18 ± 0.54	64	155
6a	$-N'(CH_2)_4NCO(C_4H_2O)NO_2$	663	0.57 ± 0.83	16	24.1
6b	$-N'(CH_2)_5NCO(C_4H_2O)NO_2$	691	1.32 ± 0.81	16	23.2
7	$-N'(CH_2)_5NCH_2C_6H_5$	592	4.11 ± 0.73	64	108.1
8a	$-N'(CH_2)_4NC_{10}H_{17}$	657	7.35 ± 0.73	4	6.09
8b	$-N'(CH_2)_4NC_{15}H_{25}$	793	11.42 ± 0.77	4	5.04
10	$-N'(CH_2)_4NCOC_6H_5$	592	1.47 ± 0.67	128	216.2
11	$-N'(CH_2)_4NCH_2C_6H_5$	565	2.61 ± 0.83	64	113.3
12	2-Pyridylmethylamino[b]	429	0.82 ± 0.40	128	298
13	$-N[(CH_2)_2OH]CH_2C_6H_5$	515	4.22 ± 0.54	128	248

MIC: Minimal Inhibitory Concentration; ND: Not determined. MW: Molecular weight. NA: Not applicable.
[a] Clog P was calculated using ACD/labs software v11.0.
[b] Connected through the amino group.

5.4.1. N-*Geranyl piperazine* (***22a***)
A yellow oil ($R_f = 0.5$, 0.52 g, 68%). ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.56 (s, 3H), 1.60 (s, 3H), 1.64 (s, 3H), 1.98–2.08 (m, 4H), 2.52 (NH proton), 2.98 (d, 2H), 5.04 (t, $J = 7.12$ Hz, 1H), 5.22 (m, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (q), 17.7 (q), 25.7 (q), 26.4 (t), 39.8 (t), 45.1 (t), 52.8 (t), 56.2 (t), 120.1 (d), 124.0 (d), 131.6 (s), 139.7 (s).

5.4.2. N-Trans–trans *farnesyl piperazine* (***22b***)
A yellow oil ($R_f = 0.6$, 0.5 g, 65%). ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.51 (s, 3H), 1.52 (s, 3H), 1.56 (s, 3H), 1.59 (s, 3H), 1.86–2.01 (m, 8H), 2.40 (NH), 2.90 (m, 2H), 4.98–5.02 (m, 2H), 5.17 (t, 1H, $J = 6.6$ Hz). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.0 (q), 16.4 (q), 17.6 (q), 25.7 (q), 26.3 (t), 26.7 (t), 39.7 (t), 39.7 (t), 45.2 (t), 53.1 (t), 56.0 (t), 56.3 (t), 120.2 (d), 123.8 (d), 124.3 (d), 131.1 (s), 135.0 (s), 139.2 (s).

5.4.3. N-*Geranyl homopiperazine* (***23a***)
A yellow oil ($R_f = 0.5$, 0.48 g, 64%). ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.47 (s, 3H), 1.50 (s, 3H), 1.55 (s, 3H), 1.69 (*sxt*, 2H), 1.88–1.98 (m, 4H), 2.52–2.55 (m, 4H), 2.82–2.88 (m, 4H), 2.98 (*d*, 2H), 3.51 (NH), 4.93–4.97 (m, 1H), 5.11–5.13 (m, 1H). [$CDCl_3$, 100 MHz]: δ_C 16.1 (q), 17.6 (q), 25.5 (q), 26.1 (t), 29.4 (t), 39.5 (t), 46.5 (t), 48.0 (t), 54.3 (t), 55.9 (t), 56.9 (t), 121.4 (d), 123.9 (d), 131.2 (s), 138.2 (s).

5.4.4. N-Trans–trans *farnesyl homopiperazine* (***23b***)
A yellow oil ($R_f = 0.6$, 0.52 g, 66%). ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.58 (s, 3H), 1.59 (s, 3H), 1.63 (s, 3H), 1.68 (s, 3H), 1.80 (sxt, 2H), 1.95–1.98 (m, 2H), 2.04–2.12 (m, 6H), 2.59 (NH), 2.63–2.68 (m, 4H), 2.92–2.98 (m, 4H), 3.11 (*d*, 2H), 5.07–5.12 (m, 2H), 5.27 (*t*, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.0 (q), 16.4 (q), 17.6 (q), 25.7 (q), 26.3 (t), 26.3 (t), 26.7 (t), 29.9 (t), 39.7 (t), 39.7 (t), 46.9 (t), 48.3 (t), 54.5 (t), 56.2 (t), 57.7 (t), 121.5 (d), 123.8 (d), 124.3 (d), 131.1 (s), 135.1 (s), 138.5 (s).

5.4.5. N-*C10 piperazine* (***25a***)
A white solid ($R_f = 0.4$, 0.46 g, 58%). ^1H NMR [$CDCl_3$, 600 MHz]; δ_H 0.83 (*t*, 3H), 1.22 (m, 14H), 1.44 (s, 2H), 1.93 (m, 1H), 2.30 (m, 2H), 2.45 (s, 4H), 2.92 (s, 4H). ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 14.1 (q), 22.6 (t), 26.5 (t), 27.5 (t), 29.3 (t), 29.5 (t), 31.9 (t), 45.1 (t), 53.2 (t), 59.1 (t).

5.4.6. N-*C15 piperazine* (***25b***)
A white solid ($R_f = 0.4$, 0.45 g, 58%). ^1H NMR [$CDCl_3$, 600 MHz]; δ_H 0.89 (t, 3H), 1.26–1.28 (m, 24H), 1.49 (s, 2H), 1.81 (s, 1H), 2.30 (m, 2H), 2.41 (s, 3H), 2.91 (s, 4H). ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 14.1 (q), 22.7 (t), 26.7 (t), 27.6 (t), 29.3 (t), 29.6 (t), 29.6 (t), 29.6 (t), 29.7 (t), 31.9 (t), 46.1 (t), 54.7 (t), 59.5 (t).

5.4.7. N-*C20 piperazine* (***25c***)
A white solid ($R_f = 0.4$, 0.48 g, 53%). ^1H NMR [$CDCl_3$, 600 MHz]; δ_H 0.85 (t, 3H), 1.22–1.24 (m, 30H), 1.45 (m, 2H), 1.99 (s, NH), 2.28 (m, 2H), 2.37 (s, 3H), 2.86 (m, 4H). ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 14.0 (t), 22.7 (t), 26.6 (t), 27.6 (t), 29.3 (t), 29.6 (t), 29.6 (t), 29.6 (t), 29.7 (t), 31.9 (t), 46.6 (t), 54.6 (t), 59.5 (t).

Table 2
BACTEC and IC_{50} results using the MDBK cell line for selected compounds.

Compound	Clog *P*	H37Rv (MIC, μM)	H37Rv (MIC, μg/mL)	XDR 194 (MIC, μM)	XDR 194 (MIC, μg/mL)	IC_{50} (μM)
6a	0.57 ± 0.83	MIC > 12.07 (SD ± 7.47)	MIC > 8 (SD ± 4.24)	NT	NT	NT
6b	1.32 ± 0.81	MIC > 11.58 (SD ± 7.16)	MIC > 8 (SD ± 4.24)	NT	NT	NT
8a	7.35 ± 0.73	1.52 > MIC > 0.76 (SD ± 0.54)	1 > MIC > 0.5 (SD ± 0.35)	3.04 > MIC > 1.52 (SD ± 0.54)	2.0 > MIC > 1.0 (SD ± 0.71)	30
8b	11.42 ± 0.77	0.63 > MIC > 0.32 (SD ± 0.22)	0.5 > MIC > 0.25 (SD ± 0.18)	1.26 > MIC > 0.63 (SD ± 0.45)	1 > MIC > 0.5 (SD ± 0.35)	24
9a	9.12 ± 0.52	NT	NT	1.5 > MIC > 0.75 (SD ± 0.53)	1 > MIC > 0.5 (SD ± 0.35)	25
SQ109[a]	6.04 ± 0.45	0.63	–	–	–	26

[a] Literature value [8]; NT: Not tested.

5.5. Synthesis of PCU piperazine isoprenyl/linear alkane

A mixture of *N*-isoprenyl piperazine/linear alkane piperazine (1.9 mmol) was reacted with (0.47 g, 0.84 mmol) PCU ditosylate (**18**) and K_2CO_3 (0.175 g, 1.27 mmol) in CH_3CN (10 mL) with reflux under nitrogen atmosphere for four days. The reaction was cooled, filtered and concentrated *in vacuo* to obtain a crude product. The residue was purified *via* column chromatography on silica gel using $CHCl_3$/MeOH/NH_4OH:88:10:2 as eluent to give the product as yellow oil.

5.5.1. N-*Geranyl piperazine PCU* (***8a***)

A yellow oil (R_f = 0.7, 0.40 g, 71%). IR ν_{max}: 2929, 1672, 1448, 1375, 1294, 1006 and 821 cm^{-1}. HRMS calculated for $C_{43}H_{68}N_4O$ ($M + H^+$) 657.5471, found 657.5446; ^1H NMR [$CDCl_3$, 400 MHz] δ_H 1.51 (J_{AB} = 10.2 Hz, 1H), 1.84 (J_{AB} = 10.2 Hz, 1H), 1.95–2.06 (m, 8H), 2.35–2.57 (m, 12H), 2.95 (d, 2H), 5.04 (t, J = 6.72 Hz, 1H), 5.22 (t, J = 6.4 Hz, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (q), 17.7 (q), 25.7 (q), 26.4 (t), 30.1 (t), 39.8 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.0 (t), 53.2 (t), 54.6 (t), 55.8 (t), 56.0 (t), 58.8 (d), 94.9 (s), 120.7 (d), 124.1 (d), 131.5 (s), 138.9 (s).

5.5.2. N-Trans–trans *farnesyl piperazine PCU* (***8b***)

A yellow oil (R_f = 0.8, 0.42 g, 63%). IR ν_{max}: 2929, 1670, 1448, 1375, 1294, 1153, 1006 and 822 cm^{-1}. HRMS calculated for $C_{53}H_{84}N_4O$ ($M + H^+$) 793.6723, found 793.6714. ^1H NMR [$CDCl_3$, 400 MHz] δ_H 1.48 (J_{AB} = 10.2 Hz, 1H), 1.84 (J_{AB} = 10.2 Hz, 1H), 1.56 (s, 3H), 1.57 (s, 3H), 1.60 (s, 3H), 1.65 (s, 3H), 1.91–2.08 (m, 10H), 2.35–2.60 (m, 12H), 2.95 (d, 2H), 5.07 (m, 2H), 5.23 (m, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.0 (q), 16.5 (q), 17.7 (q), 25.7 (q), 26.4 (t), 26.7 (t), 30.1 (t), 39.7 (t), 39.8 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 53.2 (t), 56.0 (t), 58.8 (d), 94.9 (s), 120.6 (d), 124.0 (d), 124.2 (d), 131.3 (s), 135.2 (s), 138.9 (s).

5.5.3. *C10-piperazine PCU* (***9a***)

A dark brown oil (R_f = 0.8, 0.41 g, 73%). IR ν_{max}: 2852, 2807, 1464, 1.295, 1161, 826 and 721 cm^{-1}. HRMS calculated for $C_{43}H_{76}N_4O$ ($M + H^+$) 665.6092, found 665.6114; ^1H NMR [$CDCl_3$, 400 MHz] δ_H 0.79 (t, J = 6.44 Hz, 3H), 1.17–1.19 (m, 14H), 1.40 (J_{AB} = 9.2 Hz, 1H), 1.42 (s, 2H), 1.77 (J_{AB} = 10.2 Hz, 1H), 1.91 (m, 2H), 2.28–2.32 (m, 3H), 2.37–2.52 (m, 11H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 14.1 (q), 22.6 (t), 26.4 (t), 27.5 (t), 29.3 (t), 29.5 (t), 29.5 (t), 31.8 (t), 41.7 (d), 43.4 (t), 44.4 (d), 47.9 (d), 52.6 (t), 54.5 (t), 58.6 (t), 58.7 (d), 94.6 (s).

5.5.4. *C15-piperazine PCU* (***9b***)

A light brown oil (R_f = 0.8, 0.48 g, 71%). IR ν_{max}: 2915, 2850, 2808, 1466, 1375, 1163, 1120, 826 and 720 cm^{-1}. MS (TOF) calculated for $C_{53}H_{96}N_4O$ ($M + H^+$) 805.7657, found 805.7698; ^1H NMR [$CDCl_3$, 400 MHz] δ_H 0.77 (m, 3H), 1.15 (s, br, 24H), 1.40 (m, 3H), 1.76 (J_{AB} = 10.0 Hz, 1H), 1.89 (3H), 2.20 (m, 2H), 2.27–2.40 (m, 11H), 2.49 (s, 2H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 14.1 (q), 22.6 (t), 26.9 (t), 27.6 (t), 29.3 (t), 29.5 (t), 29.6 (t), 29.6 (t), 30.1 (t), 31.9 (t), 41.8 (d), 43.4 (t), 44.2 (d), 47.9 (d), 53.2 (t), 54.6 (t), 58.8 (d), 58.8 (t), 94.7 (s).

5.5.5. *C20- piperazine PCU* (***9c***)

A yellow solid (R_f = 0.8, 0.58 g, 73%). IR ν_{max}: 2915, 2849, 2809, 1469, 1375, 1163, 1119, 827 and 718 cm^{-1}. MS (TOF) calculated for $C_{63}H_{116}N_4O$ ($M + H^+$) 945.9222, found 945.9210; ^1H NMR [$CDCl_3$, 400 MHz] δ_H 0.86 (m, 3H), 1.22 (s, br, 30H), 1.45 (m, 3H), 1.84 (J_{AB} = 10.2 Hz, 1H), 1.96 (t, 2H), 2.27–2.56 (m, 15H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 14.1 (t), 22.7 (t), 26.8 (t), 27.6 (t), 29.4 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 30.1 (t), 31.9 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 53.2 (t), 54.6 (t), 58.8 (d), 58.8 (t), 94.8 (s).

5.6. N-*Benzoyl piperazine PCU* (***10***)

A mixture of *N*-benzoyl piperazine (**26**, 0.7 g, 3.68 mmol) and PCU ditosylate (**18**, 0.93 g, 1.67 mmol) and K_2CO_3 (0.28 g, 2.0 mmol) in CH_3CN (20 mL) was refluxed for four days under N_2 atmosphere. The reaction mixture was cooled, filtered and concentrated *in vacuo*. The crude residue was purified *via* column chromatography on silica gel using CH_3Cl:MeOH:NH_4OH (88:10:2, R_f = 0.75) as eluent to give the product as a yellow oil (0.84 g, 85%). IR ν_{max}: 3463, 2952, 1623, 1430, 1292 and 723 cm^{-1}. HRMS calculated for $C_{37}H_{44}N_4O_3$ ($M + H^+$) 593.3492, found 593.3505. ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.50 (J_{AB} = 10.3 Hz, 1H), 1.85 (J_{AB} = 10.3 Hz, 1H), 2.36–2.59 (m, 10H), 3.42 (s, 2H), 3.78 (s, 2H) and 7.37 (s, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.8 (t), 41.8 (d), 42.0 (t), 43.5 (t), 44.4 (d), 47.6 (t), 48.0 (d), 52.9 (t), 94.7 (s), 127.0 (d), 128.1 (d), 128.5 (d), 135.7 (s), 170.3 (s).

5.7. N-*Benzyl piperazine PCU* (***11***)

N-Benzoyl piperazine PCU (**10**, 1 g, 1.7 mmol) was added to a stirred suspension of lithium aluminium hydride (0.25 g, 6.8 mmol) in dry THF under nitrogen. The solution was refluxed for 36 h under N_2. The solution was diluted with diethyl ether and the excess LAH was quenched by the dropwise addition of saturated aqueous Na_2SO_4. The solution was filtered, dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo* to afford a residue which was purified *via* column chromatography on silica gel using CH_3Cl:MeOH:NH_4OH (88:10:2, R_f = 0.62) as eluent to give the product as a yellow oil (55%). IR ν_{max}: 2948, 1656, 1149 and 734 cm^{-1}. HRMS calculated for $C_{37}H_{48}N_4O$ ($M + H^+$) 565.3906, found 565.3911. ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.48 (J_{AB} = 10.3 Hz, 1H), 1.84 (J_{AB} = 10.3 Hz, 1H), 1.96 (t, J = 7.92 Hz, 2H) 2.34–2.56 (m, 10H), 3.47 (s, 2H) and 7.2–7.28 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.9 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 54.6 (t), 58.8 (d), 63.0 (t), 94.8 (s), 127.0 (d), 128.2 (d), 129.3 (d), 138.0 (s).

5.8. Synthesis of 2-(aminomethyl) pyridine PCU (***12***)

A mixture of 2-(aminomethyl) pyridine (**27**, 1 g, 9.2 mmol) and of benzaldehyde (**29**, 0.98 g, 9.2 mmol) in ethanol (15 mL) was

stirred for 1 h at room temperature under nitrogen atmosphere, the corresponding imine was reduced with solid $NaBH_4$ (0.7 g, 18 mmol) which was added slowly over 30 min, the mixture was further stirred for an additional 30 min, the mixture was then refluxed overnight. The mixture was allowed to cool to RT, an additional ethanol (15 mL) was added to the reaction vessel after which 10% HCl was added to quench excess $NaBH_4$. The acidic mixture was basified with 25% aqueous ammonia solution. The desired product was extracted with dichloromethane (2×50 mL) and the solution was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was distilled under vacuum to afford (1.2 g, 65%) *N*-benzyl-*N*-{(pyridin-2-yl)methyl}amine (**30**) [21,22]. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H: 2.25 (NH), 3.84 (s, 2H), 3.92 (s, 2H), 7.14 (t, 1H), 7.26 (d, 1H), 7.30–7.37 (m, 5H), 7.62 (t, 1H), 8.56 (d, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 53.4 (t), 54.4 (t), 121.8 (d), 122.2 (d), 126.9 (d), 128.1 (d), 128.3 (d), 136.3 (d), 140.0 (d), 149.2 (s), 156.7 (s).

In the next step, a mixture of *N*-benzyl-*N*-{(pyridin-2-yl)methyl}amine (**30**, 1.5 g, 7.6 mmol), PCU ditosylate (**18**, 1.9 g, 3.4 mmol) and Et_3N (710 μL, 5.1 mmol) in CH_3CN (20 mL) was refluxed under nitrogen for four days. The reaction was monitored on TLC, after completion the reaction was filtered and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using CH_3Cl:MeOH: NH_4OH (88:10:2, $R_f = 0.8$) as eluent to give *N*-benzyl-*N*-(2-methylpyridine) PCU (**32**) (1.55 g, 75%). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.39 ($J_{AB} = 10.2$ Hz, 1H), 1.73 ($J_{AB} = 10.2$ Hz, 1H), 2.01 (t, $J = 7.8$ Hz, 2H), 2.18–2.58 (m, 8H), 3.61 (s, 2H), 3.72 (s, 2H), 7.10 (t, $J = 5.2$ Hz, 1H), 7.19–7.33 (m, 5H), 7.52 (d, $J = 7.7$ Hz, 1H), 7.60 (t, $J = 7.5$ Hz, 1H), 8.47 (d, $J = 4.2$ Hz, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 30.0 (t), 41.6 (d), 43.4 (t), 44.3 (d), 47.8 (d), 50.1 (t), 58.4 (t), 58.7 (d), 60.1 (t), 94.8 (s), 121.8 (d), 122.8 (d), 126.9 (d), 128.2 (d), 128.8 (d), 136.4 (d), 139.4 (s), 148.8 (d), 160.4 (s).

A mixture of (1.8 g, 1.7 mmol) *N*-benzyl-*N*-{(pyridin-2-yl)methyl}amine PCU (**32**), ammonium formate (0.54 g, 8.5 mmol) and 150 mg of 10% Pd/C in methanol was refluxed under nitrogen atmosphere for 15 h [27]. The mixture was cooled to RT, filtered and solution concentrated. The residue obtained was made alkaline with $NaHCO_3$ and extracted with $CHCl_3$. The was mixture dried over Na_2SO_4, concentrated *in vacuo* to afford pure 2 (aminomethyl) pyridine PCU (**12**) ($CHCl_3$:MeOH:NH_4OH, 88:10:2, $R_f = 0.65$, 0.85 g, 67%). IR ν_{max}: 3314, 2955, 1665, 1590, 1433 and 753 cm^{-1}. HRMS calculated for $C_{27}H_{32}N_4O$ ($M + H^+$) 429.2654, found 429.2667. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.48 ($J_{AB} = 10.2$ Hz, 1H), 1.83 ($J_{AB} = 10.2$ Hz, 1H), 2.01 (t, $J = 7.28$ Hz, 2H), 2.3–2.56 (m, 4H), 2.76 (m, 2H), 3.89 (s, 2H), 7.12 (t, 1H), 7.30 (d, 1H), 7.60 (t, 1H), 8.51 (d, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 32.5 (t), 41.5 (d), 43.5 (t), 44.2 (d), 46.1 (t), 47.9 (d), 55.1 (t), 58.5 (d), 95.4 (s), 122.0 (d), 122.2 (d), 136.5 (d), 149.2 (d), 159.3 (s).

5.9. Synthesis of N-benzyl ethanolamine PCU (*13*)

A mixture of benzaldehyde (**29**, 2.1 g, 20 mmol) and *N*-ethanolamine (**28**, 1.2 g, 20 mmol) in methanol (20 mL) was stirred at 25 °C under N_2 atmosphere for 2 h. The mixture was cooled to 0 °C using an external ice–salt bath after which $NaBH_4$ (1.5 g, 40 mmol) was added slowly. The mixture was stirred overnight at RT. Excess $NaBH_4$ was quenched by adding 10% HCl (20 mL), the mixture was basified with 25% NH_4OH and the product was extracted from the mixture with dichloromethane (2×30 mL). The solvent was dried over Na_2SO_4 and concentrated *in vacuo* to afford pure 2-(Benzylamino) ethanol (**31**) (2.2 g, 72%). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 2.71 (t, 2H), 3.04 (NH), 3.60 (t, 2H), 3.75 (s, 2H), 7.23–7.33 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C: 50.6 (t), 53.4 (t), 60.6 (t), 127.0 (d), 128.1 (d), 128.3 (d), 139.6 (s).

A mixture of 2-(Benzylamino) ethanol (**31**, 1.2 g, 7.9 mmol), PCU ditosylate (**18**, 2 g, 3.6 mmol) and K_2CO_3 (0.745 g, 5.4 mmol) in CH_3CN (50 mL) was refluxed under nitrogen for four days. The reaction was monitored using TLC. After completion the reaction was filtered and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using CH_3Cl:MeOH:NH_4OH (88:10:2, $R_f = 0.7$) as eluent to give 2-(benzylamino)ethanol PCU (**13**, 1.3 g, 70%). IR ν_{max}: 3371, 2951, 1601, 1452, 1043, 732 and 697 cm^{-1}. HRMS calculated for $C_{33}H_{42}N_2O_3$ ($M + H^+$) 515.3274, found 515.3288. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.44 ($J_{AB} = 10.2$ Hz, 1H), 1.77 ($J_{AB} = 10.2$ Hz, 1H), 1.96 (t, $J = 7.36$ Hz, 2H), 2.25–2.64 (m, 8H) 3.54 (t, 2H), 3.61 (s, 2H), 7.27–7.28 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.3 (t), 41.5 (d), 43.4 (t), 44.1 (d), 47.7 (d), 49.6 (t), 55.0 (t), 58.4 (t), 58.4 (d), 58.7 (t), 95.1 (s), 127.1 (d), 128.3 (s), 129.0 (s).

5.10. Biological testing

5.10.1. Broth macrodilution method

All mycobacterial work was carried out in a Level III Biosafety laboratory. All synthesized novel "cage" compounds were evaluated in triplicate against *M. tuberculosis* H37Rv. Each PCU amine compound was dissolved in methanol to prepare a final concentration of 12.8 mg/mL and dilution a 100 fold with 7H9 broth medium to give a stock concentration of 128 μg/mL. EMB was dissolved in DMSO and water similarly to serve as a standard. These was diluted two fold in sterile 30 mL universal tubes containing Middlebrook 7H9 broth supplemented with casitone, glycerol and 10% OADC enrichment to give a concentration range of 128 μg/mL–0.125 μg/mL. Tubes containing Middlebrook 7H9 broth without compounds served as the compound free controls while Middlebrook 7H9 broth containing solvents alone served as controls to monitor their inhibitory effect.

A standardized inoculum was prepared by vortexing a log phase culture of *M. tuberculosis* H37Rv in a sterile tube containing 4.5 mL phosphate buffer, 0.05% Tween 80 and 4–6 glass beads (5 mm diameter). After allowing the clumps to settle, the supernatant was aspirated and adjusted to a MacFarland number 1 standard, equivalent to 1×10^7 colony forming units (CFU)/mL. This was diluted in 7H9 broth to obtain a concentration of 1×10^5 CFU/mL. Of this, 500 μL was added into each tube of broth containing the diluted test compound concentrations, the compound free controls and the solvent controls. Colony counts of the test inoculum were prepared by plating out 20 μL onto Middlebrook 7H11 agar plates. Plates and tubes were incubated aerobically at 37 °C for twenty one days. Macroscopic readings of the tubes were documented every seven days until twenty one days.

5.10.2. BACTEC 460 TB analysis

M. tuberculosis reference strain H37Rv (ATCC 25618) and XDR strain × 194 (drug sensitivity: Isoniazid > 0.2 μg/mL; Rifampicin > 1.0 μg/mL; EMB > 2.5 μg/mL; Kanamycin > 5.0 μg/mL; Ofloxacin > 2.0 μg/mL; Amikacin > 5.0 μg/mL) were cultured in Middelbrook 7H9 medium [28], enriched with OADC (0.005%, 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) [24]. Incubation was done with continuous stirring at 37 °C. Reproducible growth (<1.0% difference) was recorded under standardized conditions. Cultures with an optical density between 0.1 and 0.6 (at 600 nm) were in exponential growth. At an optical density of approximately 0.16, 0.1 mL of culture was inoculated into a BACTEC vial (Becton Dickinson, Franklin Lakes, NJ, USA) and incubated at 37 °C to a growth index (GI) of 500 (+/− 50). This culture was used as the primary culture for drug testing. The growth index is a quantitative determination of radioactive CO_2 on a scale from 0 to 999. Drug compounds were diluted with methanol (filter sterilized through Millex LG syringe driven filters, Millipore). Compound concentrations in BACTEC vials

ranged between 8 μg/mL and 0.03125 μg/mL (final concentration). Growth index results were recorded every 24 h. Growth rates of the mycobacterial strains were calculated as daily growth index difference (ΔGI) where a ΔGI >10 was considered positive growth. Purity of *M. tuberculosis* cultures was monitored by plating onto 7H11 mycobacterium agar plates [24].

5.11. Cytotoxicity analysis

5.11.1. Materials

The RPMI 1640 (with 25 mM HEPES and L-glutamine), penicillin/streptomycin solution, and trypsin-versene mixture were purchased from Lonza and the heat-inactivated foetal bovine serum (FBS) was obtained from Invitrogen. Cytotoxicity was assessed using the Promega CellTiter 96 non-radioactive cell proliferation assay and an EL × 800 automated microplate reader from Bio-Tek Instruments, Inc.

5.11.2. Methods

The toxicities of the compounds were determined on the MDBK (Miadin Darby bovine kidney epithelium) cell line (supplied by the Department of Biochemistry, University of Kwa-Zulu Natal, South Africa). The cells were grown in a 37 °C incubator as a monolayer in RPMI 1640 supplemented with 10% (v/v) heat-inactivated FBS and penicillin/streptomycin at a final concentration of 0.1 mg/mL. Cells were then trypsinised and plated at a density of 5×10^4 cells per well into a 96-well plate and incubated for 6 h at 37 °C. Media was removed from all wells and replaced with fresh media. The test compounds were dissolved in 1% methanol with the aid of sonication. Dilutions of the test compounds were then prepared using fresh media before addition to the cells. The control untreated cell culture was incubated in fresh media containing an appropriate volume of the 1% methanol solution. This was included as a 100% viability internal control. All test samples were done in quintuplicate and repeated twice on different days. Cells were incubated with the test samples for 42 h at 37 °C. Cell viability was assessed on the basis of the conversion of the tetrazolium salt {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide} (MTT) into purple formazan crystals which can be dissolved using acidified isopropanol and then spectrophotometrically analyzed. The Promega CellTiter 96 non-radioactive cell proliferation assay was performed as per manufacturer's instructions. Briefly, MTT was added to each well and the plate incubated at 37 °C for 3 h before the formazan crystals were dissolved and the absorbance read at 570 nm. Percent cytotoxicity was calculated as follows: percent cytotoxicity = 100 − [(OD of sample/OD of control) × 100%]. All standard deviations were below 10%.

Acknowledgements

We thank the National Research Foundation, Gun 2073251, Aspen Pharmacare and the University of KwaZulu-Natal for financial support. We also thank Mr. M. Mthethwa, Department of Medical Microbiology; University of KwaZulu-Natal for providing technical assistance during the BMM biological testing of the compounds.

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