Indian Journal of Chemistry
Vol. 52B, November 2013, pp 1441-1450

Synthesis and antibacterial/antitubercular activity evaluation of symmetrical *trans*-cyclohexane-1,4-diamine derivatives

Nitin Kumar[a], Ekta Kapoor[b], Ramandeep Singh[c], Saqib Kidwai[c], Widuranga Kumbukgolla[d], Sunita Bhagat[b] & Diwan S Rawat*[a]

[a]Department of Chemistry, University of Delhi, Delhi 110 007, India

[b]Atma Ram Sanatan Dharma College, Delhi 110 021, India

[c]Vaccine and Infectious Disease Research Centre,
Translational Health Science and Technology Institute, Gurgaon 122 016, India

[d]Deparment of Biochemistry Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka, Mihintale

E-mail: dsrawat@chemistry.du.ac.in

Received 23 April 2013; accepted (revised) 10 September 2013

A library of symmetrical *trans*-cyclohexane-1,4-diamine derivatives have been synthesized and evaluated for their activity against the *M. tb* $H_{37}Rv$ strain. Most of the synthesized compounds show moderate to weak activity against *M. tb* $H_{37}Rv$ strain. Out of twenty-seven compounds tested, four compounds having substitution at *p*-position on the aromatic ring exhibit activity with MIC_{99} value ranging from 12.5 - 25 μM. Compound **9u** having *i*-propyl group substitution at *p*-position is found to be the most potent among all the tested compounds with MIC_{99} value of 12.5 μM against *M. tb* $H_{37}Rv$ strain. All these compounds have also been tested against *Methicilin* resistant *Staphylococcus aureus* (MRSA), and four of the compounds **9c, 9i, 9p** and **9s** possess good antibacterial activity with IC_{50} ranging from 128 mg/L – 256 mg/L.

Keywords: Bromhexine, *trans*-cyclohexane-1,4-diamine, *Mycobacterium tuberculosis*, tuberculosis, methicillin resistant *Staphylococcus aureus* (MRSA)

As microorganisms have developed new mechanisms of resistance and rapidly spread genes encoding them *via* mobile genetic elements; mostly plasmids and integrons, therefore the number of effective antibacterial agents has diminished and there is a need to develop better therapeutic agents[1]. The treatment of infectious diseases remains an important issue because of a combination of factors including emerging newer infectious diseases and increasing number of multidrug resistant microbial pathogens. Strains that are resistant to at least isoniazid (INH) and rifampin (RFP) are referred as multidrug-resistant TB (MDR TB) while XDR-strains are strains that in addition to INH and RFP also acquire resistance against atleast one of the three injectable second-line drugs like amikacin, kanamycin or capreomycin and a fluoroquinolone. MDR TB and XDR TB make the problem increasingly more complex and particularly pronounced for the Gram-positive bacteria, for which little effective strategy was confirmed[2,3]. In both MDR-TB and XDR-TB, the main mechanism of resistance involves efflux pumps, expelling of the drugs or antibiotics by membrane transport proteins before they reach their intracellular targets[4]. Most of the TB bacterial strain has developed resistance to the different classes of antibacterials such as β-lactams, macrolides, quinolones, glycopeptides, oxazolidinones, *etc*. and by 2003, over 59% resistant isolates have been registered[5]. The risk, however, radically increases if the host immune system is suppressed. According to WHO, tuberculosis caused by the bacterium *Mycobacterium tuberculosis* (M. tb) leads to an estimated 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million deaths from TB[6].

In the early 20th century, introduction of streptomycin in modern chemotherapy has played a significant role in mankind's fight against tuberculosis (TB)[7]. The current therapy includes combination of four drugs, *i.e.* isoniazid, rifampicin, ethambutol and pyrazinamide. Despite its efficacy to kill actively replicating *M.tb*, the pathogen has not been eradicated[8]. The resistance to fluoroquinolones (FQ) complicated the problem and situation has further worsened due to emergence of extensively-

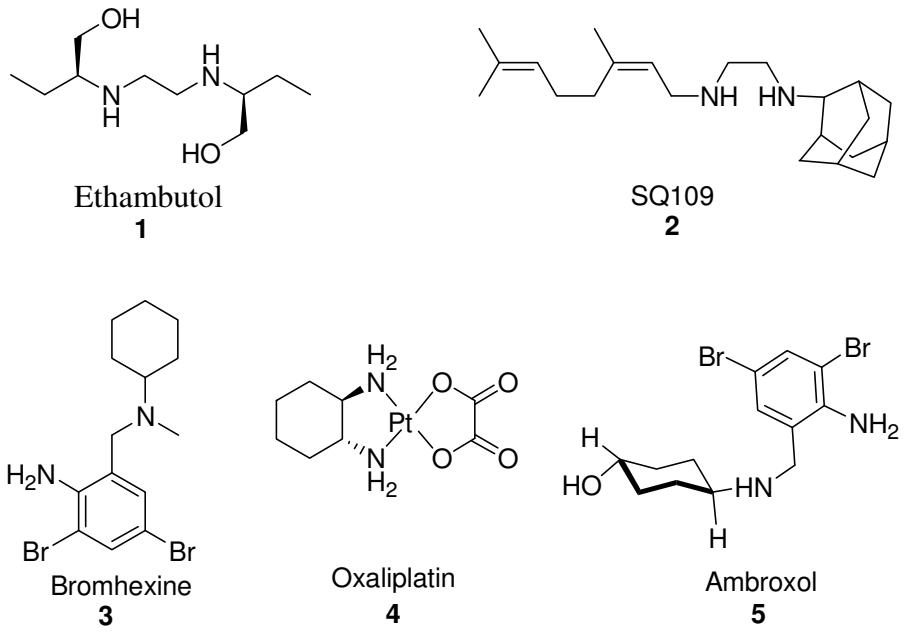
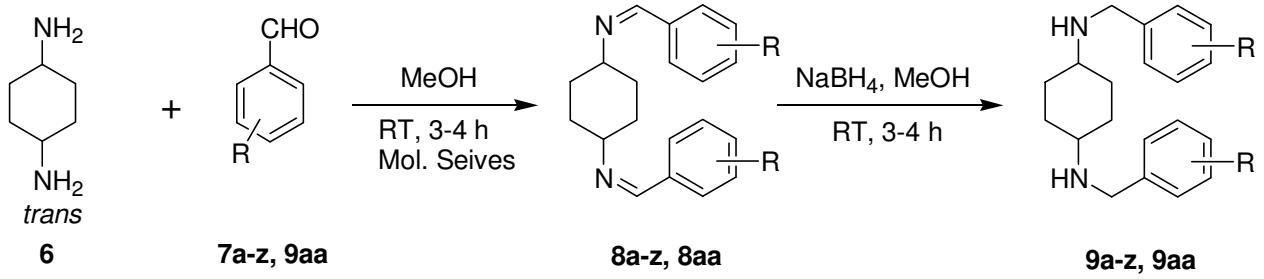


Figure 1 — Diamine based drugs

drug resistant (XDR) *M.tb.* which has caused new outbreaks of the disease at the start of the 21st century[9]. Moreover, the AIDS pandemic has aggravated the situation, as HIV infected individuals are more prone to TB infection, as compared to non-HIV infected subjects in whom the risk is estimated to be 10% on a lifetime basis. This is primarily due to drug interactions between antiretroviral drugs and anti-tuberculosis drugs which limit the treatment possibilities[10-12]. In the development of new therapies against TB, one of the effective strategies is to target essential biosynthetic pathways of the microorganism that are absent in humans. Recently, acetohydroxy acidsynthase has been identified as an attractive target for design of new generation anti-TB agents[13]. AHAS catalyses the conversion of two molecules of pyruvate to 2-acetolactate and CO_2, which plays an important part in biosynthesis of the branched chain amino acids by higher plants, algae, fungi, bacteria, and animals and there are no homologs in human and animals[14]. Ethambutol [(2*S*, 20*S*)-2,20-(ethane-1,2-diyldiimino) dibutan-1-ol] **1**, a diamine based compound in combination with other anti-TB drugs has been used as a drug of choice for the treatment of MDR-XDR-TB[15,16]. In another study 63238 compounds, based on the ethambutol structural motif were screened for antitubercular avtivity and one compound SQ109 (N-geranyl-N′-(2-adamantyl) ethane-1,2- diamine) **2**, was found to be the most potent with MIC 0.7– 1.56 μM and is currently undergoing phase II clinical trials[17]. In a recent study, it has been shown that SQ-109 targets the MmpL3 protein, an enzyme involved in the transport of lipids into the cell wall core of *M. tb*[18]. Compounds based on bromhexine **3** scafolds have also exhibited excellent anti-tubercular activity[19]. Dimers of bromhexine derivatives based on 1,2-diaminocyclohexanes (DACHs), have been widely used as a carrier ligand in the development of *cis-platin* based anticancer molecules[20]. Cyclohexane moiety has played an important role in many organic transformations[21,22] and it has been part of many biologically active compounds such as oxaliplatin **4** (Ref 23), bromhexine **3** (Ref 24), and ambroxol **5** (Ref 25) **(Figure 1)**. Oxaliplatin, {(1*R*,2*R*)-cyclohexanediamine}oxalatoplatinum(II) **4**, is a cyclohexane-1,2-diamine based platinum complex which has been approved by the FDA for the first line treatment of colorectal carcinoma in 2004[26]. Bromhexine [N-2-amino-3,5-dibromobenzyl)-N-cyclohexylmethyl-amine] **3** is another compound based on the cyclohexane motif, a semi-synthetic derivative of the alkaloid vasicine[27] used for the treatment of asthma and tuberculosis.

Methicillin resistant *Staphylococcus aureus* (MRSA) is also a global therapeutic challenge because of rapid emergence of antibiotic resistant strains. MRSA strains are resistant against β-lactams, erythromycin, gentamicin and chloramphenicol and are only susceptible to killing by vancomycin. However, vancomycin resistant strains have also been



Scheme I

Table I — Anti-tubercular activity of *trans*-1,4-diamine cyclohexane

Compd	R	$H_{37}Rv$ (MIC_{99}/μM)	ClogP
9a	4-OMe	>50	3.566
9b	4-Et	ND	5.784
9c	2-Me	>50	4.626
9d	2,3 Me	50	5.524
9e	4-Me	>50	4.726
9f	3-NO_2	>50	3.214
9g	2-CF_3	>50	5.494
9h	2,6-Me	>50	5.524
9i	2-Br	>50	5.454
9j	4-*n*-But	ND	7.9
9k	3-Br	ND	5.454
9l	3-F	ND	4.014
9m	4-F	>50	4.014
9n	3,5 CF_3	50	7.26
9o	2,4 CF_3	>50	7.26
9p	4-Cl	25	5.154
9q	4-*n*-Pr	ND	6.842
9r	H	>50	3.728
9s	4-CF_3	25	5.494
9t	3-Me	>50	4.726
9u	4-*i*-Pr	12.5	6.582
9v	2-F,4-CF_3	50	5.78
9w	4-Br	25	5.454
9x	3,4 OMe	>50	3.044
9y	4-*t*-But	25	7.38
9z	2,5 CF_3	>50	7.26
9aa	2-F	>50	4.014
INH		0.78	
RIF		<0.05	

reported recently[28]. MRSA causes skin infections, respiratory tract infections, blood stream infections and soft tissue infections. Significantly, in Intensive Care Units (ICUs) MRSA has become the major problem, causing life threatening conditions such as sepsis[29]. The results of several studies have shown that MRSA infections are more costly to manage because of increased cost for infection prevention, extensive investigations and expensive therapies[30]. Therefore, new chemotherapeutic agents are urgently needed to combat MRSA.

Recently, the synthesis of a series of symmetrical and asymmetrical cyclohexane-1,2-diamine[31,32] and cyclohexane-1,3-diamine derivatives[33] have been reported and most of these compounds have shown excellent antibacterial activity against Gram-positive and Gram-negative bacteria with low toxicity. The antimycobacterial activity of the best active symmetrical and asymmetrical derivatives against *M. tb* $H_{37}Rv$ lay in the range 3.125–12.5 mM[34]. These compounds belong to the diamine class of antitubercular drugs amongst which ethambutol and SQ 109 are the representative examples. Encouraged by these observations and as a part of the ongoing work toward the development of novel antimicrobials[35-38], a series of symmetrical cyclohexane-1,4-diamine compounds have been synthesized and screened for their antitubercular and anti-MRSA activity.

Results and Discussion

Mono-functionalized symmetrical or unsymmetrical diamines are important intermediates for the synthesis of many biologically important pharmacophores[21,22]. Synthesis of the title compounds was accomplished as outlined in **Scheme I**. In a typical reaction, substituted benzaldehyde (2 mol) and diamine (1 mol) in dry methanol was stirred, which led to the formation of the desired imines **(8a-z, 8aa)**. The isolated imines were reduced to amines **(9a-z, 9aa)** by the use of sodium borohydride, and purified over silica gel. All the compounds were characterized by standard analytical and spectroscopic methods.

In vitro antitubecular activity

In our ongoing research, diamine based compounds have been synthesized and screened against the *M. tb* $H_{37}Rv$ strain. Rifampicin and isoniazid, used as reference drugs, inhibited mycobacterial growth at a concentration of <0.05 and 0.78 μM respectively. As shown in **Table I**, most of the synthesized compounds were inactive against *M. tb* even at 50 μM concentration. In the present study, seventeen compounds have shown moderate activity against the *M. tb* $H_{37}Rv$ strain. Compounds **9p, 9s, 9w, 9y** (MIC_{99} = 25 μM) having Cl, CF_3, Br and *t*-But groups at *p*-position on the aromatic ring have exhibited moderate activity. Compound **9u** having *i*-propyl group at *p*-position exhibited maximum antitubercular activity against the *M. tb* $H_{37}Rv$ strain in this series with a MIC_{99} value of 12.5 μM.

In vitro anti MRSA activity

Thirty highly resistant MRSA strains were selected, having MIC values >128 mg/L against oxacillin. Out of the 26 compounds screened against MRSA, 4 compounds, *viz.* **9c, 9i, 9p** and **9s** showed moderate level of anti-MRSA activity. The MIC values of those compounds against 30 MRSA strains fell in the range of 128-256 mg/L. The other compounds did not show inhibitory activity against MRSA even at 1000 mg/L concentration of the compounds which were used for initial screening.

Experimental Section

All of the chemicals used in the syntheses were purchased from Sigma–Aldrich and were used as received. Thin layer chromatography (Merck TLC silica gel 60 F254) was used to monitor the progress of the reactions. The compounds were purified by silica gel column (60–120 mesh). Melting points were determined on an EZ-Melt automated melting point apparatus, Stanford Research Systems, and are uncorrected. IR (KBr) spectra were recorded using a Perkin-Elmer FT-IR spectrophotometer and the values are expressed in cm^{-1}. Mass spectral data were recorded in a Thermo Finnigan LCQ Advantage max ion trap mass spectrometer. ^1H NMR spectra were recorded on a BrukerSpectrospin spectrometer at 300 MHz and a Jeol ECX Spectrospin 400 MHz instrument while the ^{13}C NMR spectra were recorded at 75.5 and 100 MHz, respectively, using TMS as an internal standard. The chemical shift values were recorded on the δ scale and the coupling constants (J) are in Hz. Elemental analysis was performed on a Carlo Erba Model EA-1108 elemental analyzer and data of C, H, and N is within 0.4% of calculated values. ClogP values were calculated using Cambridge Soft Chem Office Ultra 10.0 software.

MIC_{99} determination against *M. tb* $H_{37}Rv$

A stock culture of *M. tb* $H_{37}Rv$ (ATCC 27294) was grown to Abs_{600nm} 0.2 in Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween 80, 0.2% glycerol, and albumin/NaCl/glucose (ADS) complex. The culture was diluted 1:1000 in 7H9-basal medium before aliquoting 50 μL into each well of a 96-well plate. Drugs were dissolved in DMSO (Sigma) to make 50 μM stock solutions. Drugs (100 mL solution) were added to the first row of the 96-well plate at a final concentration of 100 μM. Two fold serial dilutions were carried out and five dilutions of each drug (50–3.125 μM) were tested for antimycobacterial activity. The drugs were diluted 1:1 by addition of 50 μL of 1:1000 diluted cultures. Rows 6 and 12 of the 96-well plate's were no-drug controls. The plates were incubated at 37°C and the MIC_{99} values were read macroscopically using an inverted plate reader after 14 days. Each measurement was recorded three independent times.

In-vitro antibacterial assays against MRSA

Thirty strains of MRSA which are highly resistant against oxacillin (MIC >128 mg/L) were randomly selected from the culture collection at the Department of Microbiology, Faculty of Medicine and Allied Sciences Rajarata University of Sri Lanka. Twenty six of above derivatives were initially screened against 3 strains of MRSA using agar well diffusion method[39]. The compounds which exhibited a clear zone around their wells were further assayed using agar plate dilution method against 30 strains of MRSA. Using agar plate dilution method, exact MIC value of active compounds was determined against each MRSA strain[40].

(N1*Z*,N4*Z*)-N1,N4-bis(4-Ethylbenzylidene)cyclohexane-1,4-diamine, 8b: To a mixture of 4-ethylbenzaldehyde (500 mg, 3.08 mmol) and molecular sieves in dry methanol (5 mL), *trans* 1,4-diaminocyclohexane (175 mg, 1.54 mmol) was added. The reaction mixture was stirred for 3-4 h. The progress of reaction was monitored by thin layer chromatography (**Scheme I**). A white solid of imine precipitated out which was filtered and washed by

methanol. Yield: 78%; m.p. 172-74°C; IR (film): 3043, 2920, 2851, 1586, 1420, 1098, 1047, 858, 820, 732 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.20 (t, *J* = 7.5 Hz, 6H), 1.77-1.82 (m, 8H), 2.63-2.68 (m, 4H), 3.19-3.34 (m, 2H), 7.21 (d, *J* = 8.0 Hz, 4H), 7.63 (d, *J* = 8.0 Hz, 4H), 8.32 (s, 2H); ESI-MS: *m/z* 347.73 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(4-Methoxybenzylidene)-cyclohexane-1,4-diamine, 8a: Yield: 79%; m.p. 203-205°C; IR (film): 2922, 2850, 1641, 1606, 1513, 1304, 1254, 1163, 1026, 826 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.79-1.83 (m, 8H), 3.18-3.13 (m, 2H), 3.83 (s, 6H), 6.90 (d, *J* =8.7 Hz, 4H), 7.66 (d, *J* =8.7 Hz, 4H), 8.29 (s, 2H); ESI-MS: *m/z* 351.56 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2-Methylbenzylidene)cyclohexane-1,4-diamine, 8c: Yield: 69%; m.p. 151-53°C; IR (film): 3064, 2922, 2854, 1631, 1601, 1483, 1455, 1441, 1283, 1080, 1025, 955, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.70-1.99 (m, 8H), 2.50 (s, 6H), 3.21-3.42 (m, 2H), 7.16-7.29 (m, 6H), 7.86-7.88 (m, 2H), 8.68 (s, 2H); ESI-MS: *m/z* 319.53 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2,3-dimethylbenzylidene)-cyclohexane-1,4-diamine, 8d: Yield: 61%; m.p. 178-80°C; IR (film): 2926, 2857, 2815, 1631, 1453, 1092, 944, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.67-1.87 (m, 8H), 2.30 (s, 6H), 2.37 (s, 6H), 3.27-3.34 (m, 2H), 7.11-7.14 (m, 2H), 7.18-7.20 (m, 2H), 7.68-7.70 (m, 2H), 8.75 (s, 2H); ESI-MS: *m/z* 347.52 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(4-Methylbenzylidene)-cyclohexane-1,4-diamine, 8e: Yield: 68%; m.p. 185-87°C; IR (film): 2927, 2857, 2835, 1638, 1609, 1452, 1391, 1303, 1216, 1088, 817, 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.65-1.92 (m, 8H), 2.38 (s, 6H), 3.21-3.35 (m, 2H), 7.20 (d, *J* = 8.0 Hz, 4H), 7.61 (d, *J* = 8.0 Hz, 4H), 8.33 (s, 2H); ESI-MS: *m/z* 319.73 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(3-Nitrobenzylidene)cyclohexane-1,4-diamine, 8f: Yield: 63%; m.p. 228-30°C; IR (film): 2920, 2850, 1519, 1351, 1075, 812, 730, 612 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.80-1.86 (m, 8H), 3.29-3.47 (m, 2H), 7.56-7.60 (m, 2H), 8.03-8.07 (m, 2H), 8.24-8.26 (m, 2H), 8.44 (s, 2H), 8.58 (s, 2H); ESI-MS: *m/z* 381.43 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2-(Trifluoromethyl)benzylidene)cyclohexane-1,4-diamine, 8g: Yield: 61%; m.p. 155-57°C; IR (film): 3447, 2925, 2857, 1637, 1489, 1450, 1318, 1280, 1161, 1127, 1110, 1059, 1035, 909, 770 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.80-1.98 (m, 8H), 3.35-3.37 (m, 2H), 7.46-7.50 (m, 2H), 7.55-7.59 (m, 2H), 7.65-7.69 (m, 2H), 8.17-8.19 (m, 2H), 8.71 (s, 2H); ESI-MS: *m/z* 427.55 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2,6-Dimethylbenzylidene)cyclohexane-1,4-diamine, 8h: Yield: 78%; m.p. 168-70°C; IR (film): 2926, 2857, 2817, 1654, 1464, 1342, 1092, 946, 768 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.86-2.00 (m, 8H), 2.38 (s, 12H), 3.40-3.48 (m, 2H), 7.02-7.04 (m, 4H), 7.11-7.13 (m, 2H), 8.65 (s, 2H); ESI-MS: *m/z* 347.53 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2-Bromobenzylidene)cyclohexane-1,4-diamine, 8i: Yield: 65%; m.p. 163-65°C; IR (film): 2923, 2894, 2855, 1628, 1590, 1465, 1430, 1270, 1081, 1025, 955, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.65-1.89 (m, 8H), 3.31-3.49 (m, 2H), 7.25-7.27 (m, 2H), 7.32-7.34 (m, 2H), 7.55-7.57 (m, 2H), 8.00-8.02 (m, 2H), 8.72 (s, 2H); ESI-MS: *m/z* 449.23 (M^++H), 451.35 (M^++2).

(N1*Z*,N4*Z*)-N1,N4-bis(4-Butylbenzylidene)cyclohexane-1,4-diamine, 8j: Yield: 69%; m.p. 152-54°C; IR (film): 3021, 2959, 2930, 2857, 1641, 1608, 1452, 1216, 1089, 830, 756, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, *J* = 7.3 Hz, 6H), 1.31-1.37 (m, 4H), 1.57-1.61 (m, 4H), 1.78-1.83 (m, 8H), 2.62 (t, *J* = 7.3 Hz, 4H), 3.24-3.26 (m, 2H), 7.20 (d, *J* = 8.0 Hz, 4H), 7.63 (d, *J* = 8.0 Hz, 4H), 8.33 (s, 2H); ESI-MS: *m/z* 403.73 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(3-Bromobenzylidene)cyclohexane-1,4-diamine, 8k: Yield: 68%; m.p. 178-80°C; IR (film): 3061, 2924, 2852, 1643, 1563, 1425, 1206, 1066, 948, 784, 683 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.71-1.94 (m, 8H), 3.27-3.29 (m, 2H), 7.25-7.29 (m, 2H), 7.52-7.54 (m, 2H), 7.55-7.61 (m, 2H), 7.93 (s, 2H), 8.30 (s, 2H); ESI-MS: *m/z* 449.21 (M^++H), 451.28 (M^++2), 453.18 (M^++4).

(N1*Z*,N4*Z*)-N1,N4-bis(3-Fluorobenzylidene)cyclohexane-1,4-diamine, 8l: Yield: 63%; m.p. 158-60°C; IR (film): 2945, 2866, 1646, 1630, 1485, 1461, 1389, 1234, 1113, 1123, 763 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.67-1.98 (m, 8H), 3.21-3.35 (m, 2H), 7.09-7.11 (m, 2H), 7.35-7.39 (m, 2H), 7.44-7.48 (m, 4H), 8.33 (s, 2H); ESI-MS: *m/z* 327.45 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(4-Fluorobenzylidene)cyclohexane-1,4-diamine, 8m: Yield: 61%; m.p. 180-82°C; IR (film): 3468, 2931, 2857, 1382. 1080, 817 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.77-1.92 (m, 8H), 3.25-3.27 (m, 2H), 7.06-7.09 (m, 4H), 7.71-7.74 (m, 4H), 8.34 (s, 2H); ESI-MS: *m/z* 327.51 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(3,5-bis(Trifluoromethyl)-benzylidene)cyclohexane-1,4-diamine, 8n: Yield: 63%; m.p. 176-78°C; IR (film): 2938, 2866, 1454, 1392, 1371, 1338, 1277, 1163, 1136, 1083, 899,700

cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.80-1.89 (m, 8H), 3.37-3.42 (m, 2H), 7.91 (s, 2H), 8.20 (s, 4H), 8.46 (s, 2H); ESI-MS: *m/z* 563.44 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2,4-bis(Trifluoromethyl)-benzylidene)cyclohexane-1,4-diamine, 8o: Yield: 63%; m.p. 168-70°C; IR (film): 2943, 2865, 2844, 1637, 1578, 1347, 1281, 1167, 1082, 1057, 913, 856, 670 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.80-1.90 (m, 8H), 3.40-3.48 (m, 2H), 7.83 (d, *J* =8.2 Hz, 2H), 7.94 (s, 2H), 8.36 (d, *J* =8.2 Hz, 2H), 8.73 (s, 2H); ESI-MS: *m/z* 563.73 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(4-Chlorobenzylidene)cyclohexane-1,4-diamine, 8p: Yield: 67%; m.p. 225-28°C; IR (film): 3023, 2927, 2858, 1635, 1619, 1576, 1461, 1310, 1256, 1090, 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.65-1.96 (m, 8H), 3.23-3.39 (m, 2H), 7.55 (d, *J* =8.2 Hz, 4H), 7.71 (d, *J* =8.2 Hz, 4H), 8.36 (s, 2H); ESI-MS: *m/z* 359.43 (M^++H), 361.78 (M^++2), 363.23 (M^++4).

(N1*Z*,N4*Z*)-N1,N4-bis(4-Propylbenzylidene)cyclohexane-1,4-diamine, 8q: Yield: 73%; m.p. 155-57°C; IR (film): 3021, 2925, 2852, 1640, 1609, 1571, 1465, 1452, 1300, 1216, 1087, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, *J* = 7.3 Hz, 6H), 1.63-1.69 (m, 8H), 1.70-1.91 (m, 4H), 2.59 (t, *J* = 7.3 Hz, 4H), 3.21-3.28 (m, 2H), 7.20 (d, *J* = 8.2 Hz, 4H), 7.63 (d, *J* = 8.2 Hz, 4H), 8.34 (s, 2H); ESI-MS: *m/z* 375.66 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-Dibenzylidenecyclohexane-1,4-diamine, 8r: Yield: 64%; m.p. 170-72°C; IR (film): 3081, 3021, 2924, 2855, 2825, 1643, 1448, 1391, 1090, 1066, 950, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.79-1.84 (m, 8H), 3.27-3.28 (m, 2H), 7.32-7.40 (m, 6H), 7.71-7.77 (m, 4H), 8.37 (s, 2H); ESI-MS: *m/z* 291.45 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(4-(Trifluoromethyl)benzylidene)cyclohexane-1,4-diamine, 8s: Yield: 68%; m.p. 186-88°C; IR (film): 2934, 2862, 1645, 1323, 1215, 1156, 1143, 1107, 1084, 1053, 835 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.62-1.98 (m, 8H), 3.29-3.44 (m, 2H), 7.65 (d, *J* =8.2 Hz, 4H), 7.84 (d, *J* =8.2 Hz, 4H), 8.42 (s, 2H); ESI-MS: *m/z* 427.53 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(3-Methylbenzylidene)-cyclohexane-1,4-diamine, 8t: Yield: 71%; m.p. 184-86°C; IR (film): 3063, 3045, 2931, 2855, 2832, 1643, 1606, 1582, 1451, 1380, 1295, 1093, 1080, 949, 790, 693 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.65-1.85 (m, 8H), 2.38 (s, 6H), 3.20-3.41 (m, 2H), 7.19-7.25 (m, 2H), 7.26-7.31 (m, 2H), 7.47-7.48 (m, 2H), 7.61 (s, 2H), 8.39 (s, 2H); ESI-MS: *m/z* 319.73 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(4-*iso* Propylbenzylidene)-cyclohexane-1,4-diamine, 8u: Yield: 72%; m.p. 182-84°C; IR (film): 3025, 2927, 2832, 1641, 1619, 1573, 1467, 1454, 1315, 1218, 1090, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.26 (d, *J* = 6.8 Hz, 12H), 1.73-1.83 (m, 8H), 2.92 (sept, 2H), 3.19-3.31 (m, 2H), 7.25 (d, *J* = 8.0 Hz, 4H), 7.65 (d, *J* = 8.0 Hz, 4H), 8.34 (s, 2H); ESI-MS: *m/z* 375.60 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2-Fluoro-4-(trifluoromethyl)benzylidene)cyclohexane-1,4-diamine, 8v: Yield: 62%; m.p. 151-53°C; IR (film): 3422, 2950, 2907, 2869, 2845, 1638, 1427, 1332, 1217, 1172, 1060, 886 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.80-1.87 (m, 8H), 3.37-3.39 (m, 2H), 7.35 (d, *J* =8.2 Hz, 2H), 7.43 (d, *J* =8.2 Hz, 2H), 8.11-8.15 (m, 2H), 8.68 (s, 2H); ESI-MS: *m/z* 463.47 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(4-Bromobenzylidene)cyclohexane-1,4-diamine, 8w: Yield: 63%; m.p. 230-32°C; IR (film): 2925, 2848, 2813, 1642, 1586, 1481, 1379, 1300, 1001, 838 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.67-1.97 (m, 8H), 3.21-3.38 (m, 2H), 7.52 (d, *J* =8.2 Hz, 4H), 7.59 (d, *J* =8.2 Hz, 4H), 8.31 (s, 2H); ESI-MS: *m/z* 447.33 (M^++H), 449.56 (M^++2), 451.38 (M^++4).

(N1*Z*,N4*Z*)-N1,N4-bis(3,4-Dimethoxybenzylidene)cyclohexane-1,4-diamine, 8x: Yield: 71%; m.p. 207-209°C; IR (film): 2944, 2858, 1631, 1616, 1517, 1314, 1259, 1170, 1126, 1087, 1009, 974, 826 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.71-1.90 (m, 8H), 3.19-3.29 (m, 2H), 3.90 (s, 6H), 3.95 (s, 6H), 6.85-6.87 (m, 2H), 7.13-7.16 (m, 2H), 7.39-7.41 (m, 2H), 8.27 (s, 2H); ESI-MS: *m/z* 411.71 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(4-*tert*-Butylbenzylidene)-cyclohexane-1,4-diamine, 8y: Yield: 73%; m.p. 242-44°C; IR (film): 2961, 2928, 2856, 1641, 1608, 1383, 1362, 1305, 1084, 945, 835, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (s, 18H), 1.78-1.83 (m, 8H), 3.24-3.26 (m, 2H), 7.41 (d, *J* = 8.2 Hz, 4H), 7.65 (d, *J* = 8.2 Hz, 4H), 8.34 (s, 2H); ESI-MS: *m/z* 403.65 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2,5-bis(Trifluoromethyl)-benzylidene)cyclohexane-1,4-diamine, 8z: Yield: 65%; m.p. 146-48°C; IR (film): 3083, 2931, 2863, 1640, 1613, 1502, 1450, 1422, 1335, 1315, 1263, 1175, 1126, 1086, 1041, 945, 843 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.66-1.90 (m, 8H), 3.45-3.49 (m, 2H), 7.72-7.76 (m, 2H), 7.80-7.82 (m, 2H), 8.56 (s, 2H), 8.71 (s, 2H); ESI-MS: *m/z* 563.43 (M^++H).

(N1*Z*,N4*Z*)-N1,N4-bis(2-Fluorobenzylidene)cyclohexane-1,4-diamine, 8aa: Yield: 73%; m.p. 142-44°C; IR (film): 2924, 2856, 1636, 1612, 1483, 1451,

1391, 1224, 1104, 1079, 763 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.68-1.98 (m, 8H), 3.31-3.34 (m, 2H), 7.06-7.09 (m, 2H), 7.16-7.18 (m, 2H), 7.36-7.38 (m, 2H), 7.96-7.98 (m, 2H), 8.67 (s, 2H); ESI-MS: *m/z* 327.53 (M^++H).

N1,N4-bis(4-Ethylbenzyl)cyclohexane-1,4-diamine, 9b:To a solution of **8b** (500 mg, 1.23 mmol) in dry MeOH (10 mL) sodium borohydride (141 mg, 3.72 mmol) was added under inert atmosphere. The mixture was stirred for 4 h at RT (**Scheme I**). Reaction was quenched by the addition of cold water and extracted with chloroform (2 × 15 mL). Organic layer was dried over anhydrous Na_2SO_4 and excess of solvent was removed under reduced pressure. Compounds were purified over silica gel to afford white solid. Yield: 78%; m.p. 75-77°C; IR (film): 3317, 3073, 2928, 2855, 1608, 1454, 1313, 1260, 1160, 1117, 1059, 1037, 768 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16-1.24 (m, 10H), 1.26-1.70 (brs, 2H), 1.96-1.98 (m, 4H), 2.59-2.65 (m, 6H), 6.76 (s, 4H), 7.13 (d, *J* = 7.8 Hz, 4H), 7.20 (d, *J* = 7.8 Hz, 4H); ESI-MS: *m/z* 351.73 (M^++H).

N1,N4-bis(4-Methoxybenzyl)cyclohexane-1,4-diamine, 9a: Yield: 83%; m.p. 111-13°C; IR (film): 3346, 2917, 2850, 1609, 1279, 1175, 1131, 923, 792, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.15-1.18 (m, 4H), 1.42-1.81 (brs, 2H), 1.96-1.97 (m, 4H), 2.49-2.61 (m, 2H), 3.73 (s, 4H), 3.79 (s, 6H), 6.84 (d, *J* =6.8Hz, 4H), 7.21 (d, *J* =6.8Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 31.28, 50.67, 55.20, 56.10, 113.75, 129.21, 132, 69, 158.50; ESI-MS: *m/z* 355.53 (M^++H).

N1,N4-bis(2-Methylbenzyl)cyclohexane-1,4-diamine, 9c: Yield: 76%; m.p. 93-95°C; IR (film,): 3417, 2927, 2403, 1605, 1443, 1246, 1082, 987, 812, 738 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.18-1.26 (m, 4H), 1.43-1.61 (brs, 2H), 2.01-2.02 (m, 4H), 2.34 (s, 6H), 2.53-2.54 (m, 2H), 3.77 (s, 4H), 7.13-7.18 (m, 4H), 7.24-7.29 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 18.88, 32.04, 49.06, 56.76, 125.92, 126.86, 128.29, 130.23, 136.11, 138.66; ESI-MS: *m/z* 323.50 (M^++H).

N1,N4-bis(2,3-Dimethylbenzyl)cyclohexane-1,4-diamine, 9d: Yield: 79%; m.p. 100-102°C; IR (film): 3345, 3049, 2914, 2852, 1617, 1501, 1444, 1430, 1398, 1273, 1225, 963, 821, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.11-1.15 (m, 4H), 1.19-1.49 (brs, 2H), 1.93-1.94 (m, 4H), 2.17 (s, 6H), 2.20 (s, 6H), 2.41-2.54 (m, 2H), 3.70 (s, 4H), 6.95-7.00 (m, 4H), 7.01-7.06 (m, 2H); ESI-MS: *m/z* 351.78 (M^++H).

N1,N4-bis(4-Methylbenzyl)cyclohexane-1,4-diamine, 9e: Yield: 86%; m.p. 72-74°C; IR (film): 3321, 3008, 2920, 2848, 2798, 1514, 1454, 1442, 1122, 1022, 806, 753, 727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 1.05-1.10 (m, 4H), 1.19-1.49 (brs, 2H), 1.88-1.90 (m, 4H), 2.25 (s, 6H), 2.41-2.42 (m, 2H), 3.68 (s, 4H), 7.04 (d, *J* = 7.8 Hz, 4H), 7.10 (d, *J* = 7.8 Hz, 4H); ESI-MS: *m/z* 323.50 (M^++H).

N1,N4-bis(3-Nitrobenzyl)cyclohexane-1,4-diamine, 9f: Yield: 68%; m.p. 122-24°C; IR (film): 3260, 2928, 2851, 2819, 1523, 1473, 1345, 1132, 1090, 811, 734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.17-1.19 (m, 8H), 1.41-1.62 (brs, 2H), 2.39-2.42 (m, 2H), 3.92 (s, 4H), 7.46-7.50 (m, 2H), 7.66-7.68 (m, 2H), 8.08-8.10 (m, 2H), 8.21 (s, 2H); ESI-MS: *m/z* 385.59 (M^++H).

N1,N4-bis(2-(Trifluoromethyl)benzyl)cyclohexane-1,4-diamine, 9g: Yield: 73%; m.p. 53-55°C; IR (film): 3361, 3074, 2928, 2855, 1608, 1584, 1455, 1365, 1312, 1261, 1158, 1059, 1037, 955, 768 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16-1.18 (m, 4H), 1.25-1.61 (brs, 2H), 1.97-1.99 (m, 4H), 2.47-2.48 (m, 2H), 3.85 (s, 4H), 6.99-7.04 (m, 2H), 7.07-7.11 (m, 2H), 7.19-7.23 (m, 2H), 7.30-7.33 (m, 2H); ESI-MS: *m/z* 431.53 (M^++H).

N1,N4-bis(2,6-Dimethylbenzyl)cyclohexane-1,4-diamine, 9h: Yield: 77%; m.p. 105-107°C; IR (film): 3283, 3066, 2921, 2853, 1588, 1467, 1121, 1093, 886, 769, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.21-1.29 (m, 4H), 1.42-1.61 (brs, 2H), 1.98-2.09 (m, 4H), 2.39 (s, 12H), 2.51-2.61 (m, 2H), 3.77 (s, 4H), 6.98-7.08 (m, 6H); ESI-MS: *m/z* 351.63 (M^++H).

N1,N4-bis(2-Bromobenzyl)cyclohexane-1,4-diamine, 9i: Yield: 74%; m.p. 84-86°C; IR (film): 3461, 2919, 2851, 1606, 1442, 1356, 1134, 1023, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.18-1.21 (m, 4H), 1.46-1.64 (brs, 2H), 1.99-2.00 (m, 4H), 2.48-2.49 (m, 2H), 3.87 (s, 4H), 7.08-7.12 (m, 2H), 7.24-7.28 (m, 2H), 7.37-7.39 (m, 2H), 7.51-7.53 (m, 2H); ESI-MS: *m/z* 453.23 (M^++H), 455.78 (M^++2), 457.58 (M^++4).

N1,N4-bis(4-Butylbenzyl)cyclohexane-1,4-diamine, 9j: Yield: 86%; m.p. 44-46°C; IR (film): 3309, 3088, 2929, 2855, 1513, 1464, 1455, 1418, 1128, 813, 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$):0.91 (t, *J* = 7.3 Hz, 6H), 1.13-1.18 (m, 4H), 1.25-1.56 (brs, 2H), 1.31-1.37 (m, 4H), 1.57-1.61 (m, 4H), 1.97-1.99 (m, 4H), 2.48-2.50 (m, 6H), 3.86 (s, 4H), 7.42 (d, *J* = 7.8 Hz, 4H), 7.56 (d, *J* = 7.8 Hz, 4H); ESI-MS: *m/z* 407.73 (M^++H).

N1,N4-bis(3-Bromobenzyl)cyclohexane-1,4-diamine, 9k: Yield: 74%; m.p. 87-79°C; IR (film): 3260,

2935, 2847, 1578, 1452, 1263, 1093, 1025, 886, 794 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.05-1.13 (m, 4H), 1.19-1.41 (brs, 2H), 1.89-1.90 (m, 4H), 2.47-2.49 (m, 2H), 3.69 (s, 4H), 7.10-7.12 (m, 2H), 7.15-7.18 (m, 2H), 7.28-7.30 (M, 2H), 7.40 (s, 2H); ESI-MS: *m/z* 451.33 (M^++H), 453.58 (M^++2), 455.42 (M^++4).

N1,N4-bis(3-Fluorobenzyl)cyclohexane-1,4-diamine, 9l: Yield: 69%; m.p. 60-62°C; IR (film): 3309, 3064, 2927, 2853, 1616, 1589, 1486, 1448, 1358, 1252, 1139, 1122, 940, 868, 782, 685 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.13-1.18 (m, 4H), 1.48-1.52 (brs, 2H), 1.97-1.98 (m, 4H), 2.41-2.44 (m, 2H), 3.80 (s, 4H), 6.90-6.95 (m, 2H), 7.03-7.08 (m, 4H), 7.24-7.30 (m, 2H); ESI-MS: *m/z* 331.73 (M^++H).

N1,N4-bis(4-Fluorobenzyl)cyclohexane-1,4-diamine, 9m: Yield: 68%; m.p. 68-70°C; IR (film): 3271, 2934, 2852, 1601, 1584, 1453, 1415, 1308, 1214, 1152, 1131, 1014, 990, 822, 734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 1.15-1.17 (m, 4H), 1.21-1.39 (brs, 2H), 1.96-1.97 (m, 4H), 2.43-2.46 (m, 2H), 3.76 (s, 4H), 6.97-7.01 (m, 4H), 7.25-7.28 (m, 4H); ESI-MS: *m/z* 331.43 (M^++H).

N1,N4-bis(3,5-bis(Trifluoromethyl)benzyl)cyclohexane-1,4-diamine, 9n: Yield: 73%; m.p. 105-107°C; IR (film): 3345, 2919, 2852, 1609, 1279, 1165, 1131, 913, 792, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.07-1.12 (m, 4H), 1.21-1.41 (brs, 2H), 1.92-1.93 (m, 4H), 2.39-2.52 (m, 4H), 3.86 (s, 4H), 7.67 (s, 2H), 7.73 (s, 2H); ESI-MS: *m/z* 567.44 (M^++H).

N1,N4-bis(2,4-bis(Trifluoromethyl)benzyl)cyclohexane-1,4-diamine, 9o: Yield: 70%; m.p. 60-62°C; IR (film): 3368, 2930, 2857, 1629, 1459, 1346, 1276, 1170, 1083, 1058, 911, 840, 690 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.06-1.09 (m, 4H), 1.23-1.39 (brs, 2H), 1.90-1.92 (m, 4H), 2.42-2.50 (m, 2H), 3.93 (s, 4H), 7.68-7.74 (m, 3H), 7.79-7.83 (m, 3H); ESI-MS: *m/z* 567.54 (M^++H).

N1,N4-bis(4-Chlorobenzyl)cyclohexane-1,4-diamine, 9p: Yield: 67%; m.p. 82-84°C; IR (film): 3291, 2937, 2851, 1489, 1450, 1407, 1127, 1084, 1014, 805, 745, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.06-1.09 (m, 4H), 1.21-1.49 (brs, 2H), 1.83-1.89 (m, 4H), 2.39-2.41 (m, 2H), 3.69 (s, 4H), 7.15 (d, *J* = 8.6 Hz, 4H), 7.19 (d, *J* = 8.6 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 31.95, 50.56, 56.17, 128.44, 129.32, 132.44, 139.27; ESI-MS: *m/z* 363.46 (M^++H), 365.46 (M^++2), 367.46 (M^++4).

N1,N4-bis(4-Propylbenzyl)cyclohexane-1,4-diamine, 9q: Yield: 79%; m.p. 48-50°C; IR (film): 3312, 2923, 2850, 1608, 1512, 1462, 1446, 1260, 1127, 1091, 816, 763, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 0.83 (t, *J* = 7.3 Hz, 6H), 1.07-1.11 (m, 4H), 1.25-1.42 (brs, 2H), 1.50-1.57 (m, 4H), 1.90-1.91 (m, 4H), 2.46-2.50 (m, 6H), 3.69 (s, 4H), 7.04 (d, *J* = 8.2 Hz, 4H), 7.13 (d, *J* = 8.2 Hz, 4H); ESI-MS: *m/z* 379.65 (M^++H).

N1,N4-Dibenzylcyclohexane-1,4-diamine, 9r: Yield: 75%; m.p. 67-69°C; IR (film): 3422, 3000, 2925, 2848, 2869, 1601, 1491, 1451, 1359, 1126, 1067, 904, 753, 731, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.11-1.21 (m, 4H), 1.25-1.39 (brs, 2H), 1.94-1.98 (m, 4H), 2.48-2.49 (m, 2H), 3.79 (s, 4H), 7.21-7.25 (m, 2H), 7.29-7.31 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.95, 51.34, 56.23, 126.80, 128.01, 128.37, 140.72; ESI-MS: *m/z* 295.51 (M^++H).

N1,N4-bis(4-(Trifluoromethyl)benzyl)cyclohexane-1,4-diamine, 9s: Yield: 68%; m.p. 92-94°C; IR (film): 3343, 2927, 2866, 1591, 1515, 1427, 1331, 1276, 1215, 1167, 1065, 978, 871, 833, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.11-1.15 (m, 4H), 1.23-1.42 (brs, 2H), 1.97-1.99 (m, 4H), 2.43-2.47 (m, 2H), 3.80 (s, 4H), 7.19-7.23 (m, 4H), 7.43-7.47 (m, 4H); ESI-MS: *m/z* 431.44 (M^++H).

N1,N4-bis(3-Methylbenzyl)cyclohexane-1,4-diamine, 9t: Yield: 88%; m.p. 66-68°C; IR (film): 3309, 3023, 2924, 2853, 1609, 1590, 1486, 1451, 1377, 1357, 1126, 1091, 1040, 779 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.07-1.12 (m, 4H), 1.28-1.61 (brs, 2H), 1.90-1.91 (m, 4H), 2.26 (s, 6H), 2.43-2.44 (m, 2H), 3.68 (s, 4H), 6.97-6.98 (m, 2H), 7.01-7.05 (m, 4H), 7.11-7.17 (m, 2H); ESI-MS: *m/z* 323.49 (M^++H).

N1,N4-bis(4-*iso*-Propylbenzyl)cyclohexane-1,4-diamine, 9u: Yield: 83%; m.p. 48-50°C; IR (film): 3310, 2957, 2850, 1512, 1459, 1124, 1053, 885, 814, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.17-1.19 (m, 4H), 1.22 (d, *J* = 6.8 Hz, 12H), 1.28-1.61 (brs, 2H), 1.97-1.99 (m, 4H), 2.51-2.53 (m, 2H), 2.88 (sept, 2H), 3.76 (s, 4H), 7.16 (d, *J* = 8.2 Hz, 4H), 7.22 (d, *J* = 8.2 Hz, 4H); ESI-MS: *m/z* 379.60 (M^++H).

N1,N4-bis(2-fluoro-4-(trifluoromethyl)benzyl)-cyclohexane-1,4-diamine, 9v: Yield: 70%; m.p. 60-62°C; IR (film): 3313, 2930, 2857, 1588, 1510, 1429, 1329, 1277, 1211, 1127, 1065, 908, 879, 835, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16-1.18 (m, 4H), 1.26-1.49 (brs, 2H), 2.46-2.48 (m, 6H), 3.90 (s, 4H), 7.27-7.30 (m, 2H), 7.37-7.39 (m, 2H), 7.49-7.53 (m, 2H); ESI-MS: *m/z* 467.42 (M^++H).

N1,N4-bis(4-Bromobenzyl)cyclohexane-1,4-diamine, 9w: Yield: 68%; m.p. 76-78°C; IR (film): 3349, 3018, 2935, 2850, 1486, 1449, 1215, 1120, 1009, 801,

747 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.13-1.16 (m, 4H), 1.21-1.42 (brs, 2H), 1.94-1.96 (m, 4H), 2.46-2.47 (m, 2H), 3.75 (s, 4H), 7.17-7.20 (m, 4H), 7.41-7.44 (m, 4H); ESI-MS: *m/z* 451.33 (M^++H), 453.26 (M^++2), 455.28 (M^++4).

N1,N4-bis(3,4-Dimethoxybenzyl)cyclohexane-1,4-diamine, 9x: Yield: 74%; m.p. 115-17°C; IR (film): 3304, 3000, 2931, 2836, 2253, 1591, 1514, 1463, 1417, 1235, 1135, 1028, 913, 807, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.14-1.19 (m, 4H), 1.25-1.45 (brs, 2H), 1.97-1.99 (m, 2H), 2.51-2.53 (m, 2H), 3.74 (s, 4H), 3.86 (s, 12H), 6.80-6.82 (m, 4H), 6.85-6.86 (m, 4H); ESI-MS: *m/z* 415.56 (M^++H).

N1,N4-bis(4-*tert*-Butylbenzyl)cyclohexane-1,4-diamine, 9y: Yield: 82%; m.p. 139-41°C; IR (film): 3315, 2929, 2889, 2849, 1625, 1580, 1483, 1454, 1383, 1354, 1280, 1225, 1216, 1174, 1134, 1080, 994, 885, 839, 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.14-1.19 (m, 4H), 1.21-1.29 (brs, 2H), 1.30 (s, 18H), 1.98-1.99 (m, 4H), 2.51-2.52 (m, 2H), 3.77 (s, 4H), 7.22 (d, *J* = 7.7 Hz, 4H), 7.32 (d, *J* = 7.7 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 31.34, 31.93, 34.40, 50.9.31, 125.30, 127.76, 137.67, 149.71; ESI-MS: *m/z* 407.73 (M^++H).

N1,N4-bis(2,5-bis(Trifluoromethyl)benzyl)cyclohexane-1,4-diamine, 9z: Yield: 71%; m.p. 95-97°C; IR (film): 3456, 2921, 2851, 2604, 1686, 1630, 1420, 1318, 1303, 1221, 978, 861, 783, 663 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.14-1.21 (m, 4H), 1.24-1.56 (brs, 2H), 2.00-2.02 (m, 4H), 2.52-2.53 (m, 2H), 4.02 (s, 4H), 7.59 (d, *J* = 7.7 Hz, 2H), 7.74 (d, *J* = 7.7 Hz, 2H), 8.03 (s, 2H); ESI-MS: *m/z* 567.51 (M^++H).

N1,N4-bis(2-Fluorobenzyl)cyclohexane-1,4-diamine, 9aa: Yield: 78%; m.p. 90-92°C; IR (film): 3305, 2928, 2879, 2848, 1615, 1582, 1485, 1455, 1383, 1364, 1260, 1225, 1206, 1174, 1131, 1080, 994, 882, 839, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.07-1.09 (m, 4H), 1.21-1.49 (brs, 2H), 1.81-1.89 (m, 4H), 2.37-2.43 (m, 2H), 3.80 (s, 4H), 7.18 (d, *J* = 8.6 Hz, 4H), 7.21 (d, *J* = 8.6 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.81, 44.65, 55.90, 115.10, 115.31, 123.99, 127.42, 127.58, 128.45, 130.21; ESI-MS: *m/z* 331.43 (M^++H).

Conclusion

Herein is reported the synthesis and antimycobacterial activity evaluation of a library of twenty seven symmetrically substituted *trans*-cyclohexane-1,4-diamines. Most of the compounds have shown moderate to good antitubercular activity (MIC_{99} = 50-25 μM). Compound **9u** has shown promising antitubercular activity against the *M. tb* $H_{37}Rv$ strain with a MIC_{99} value of 12.5 μM. Four of the compounds also showed moderate inhibitory activity against MRSA.

Acknowledgement

DSR thanks the University Grants Commission ([F. No. 41 202/2012 (SR)]), New Delhi, India, and University of Delhi, Delhi, India for financial support. NK and EK both contributed equally to this study. NK is thankful to CSIR for the award of senior research fellowships. RS acknowledges the financial support provided by the Department of Biotechnology, India. Authors are thankful to CIF-USIC, University of Delhi, Delhi for NMR spectral data and RSIC, CDRI, Lucknow for mass spectra. The authors are also thankful to Prof. Anil K.Tyagi, University of Delhi for access to the BSL-3 facility.

References

1 Michalska K, Karpiuk I, Król M & Tyski S, *Bioorg Med Chem*, 21, **2013**, 577.
2 Koul A, Arnoult E, Lounis N, Guillemont J & Andries K, *Nature*, 469, **2011**, 483.
3 Bondock S, Naser T & Ammar Y A, *Eur J Med Chem*, DOI 10.1016/j.ejmech.**2012**.12.050.
4 Saidijam M, Benedetti G, Ren Q, Xu Z, Hoyle C J, Palmer S L, Ward A, Bettaney K E, Szakonyi G, Meuller J, Morrison S, Pos M K, Butaye P, Walravens K, Langton K, Herbert R B, Skurray RA, Paulsen I T, O'Reilly J, Rutherford N G, Brown M H, Bill R M & Henderson P J F, *Curr Drugs Targets*, 7, **2006**, 793.
5 National Nosocomial Infections Surveillance (NNIS) system report. Data summary from January 1992 through June 2004, issued on October 2004, *Am J Infect Control*, 32, **2004**, 470.
6 WHO report on Global Tuberculosis Report **2012**.
7 Burman W J & Winkle R V, *Eur Respir J*, 37, **2011**, 441.
8 Yew W W, Lange C & Leung CC, *Int J Tuberc Lung Dis*, 13, **2009**, 1320.
9 Kaur D, Guerin M E, Skovierová H, Brennan P J & Jackson M, *Proc Natl Acad Sci* (*USA*), 107, **2010**, 12275.
10 Menzies D, Al Jahdali H & Al Otaibi B, *Indian J Med Res*, 133, **2011**, 257.
11 Marks S M, Magee E & Robison V, *Int J Tuberc Lung Dis*, 15, **2011**, 465.
12 Yew W W, Cynamon M & Zhang Y, *Expert Opin Emerg Drugs*, 16, **2011**, 1.
13 Grandoni J A, Marta P T & Schloss J V, *J Antimicrob Chemother*, 4, **1998**, 475.
14 Chipman D, Barak Z & Schloss J V, *Biochim Biophys Acta*, 2, **1998**, 401.
15 Beena & Rawat D S, *Med Res Rev*, DOI: 10.1002/med.21262.
16 Takayama K & Kilburn J O, *Antimicrob Agents Chemother*, 33, **1989**, 1493.
17 Chen P, Gearhart J, Protopopova M, Einck L & Nacy C A, *J Antimicrob Chemother*, 58, **2006**, 332.

18 Tahlan K, Wilson R, Kastrinsky D B, Arora K, Nair V, Fischer E, Barnes S W, Walker J R, Alland D, Barry C E & Boshoff H I, *Antimicrob Agents Chemother*, 56, **2012**, 1797.
19 Gautam R, Saklani A & Jachak S M, *J Ethnopharmacol*, 110, **2007**, 200.
20 Peritz A, Al-Baker S, Vollano J F, Schurig J E, Bradner W T & Dabrowiak J C, *J Med Chem*, 33, **1990**, 2184.
21 Noji M, Okamoto K & Kidani Y, *J Med Chem*, 24, **1981**, 508.
22 Jacobsen E N, Zhang W, Muci A R, Ecker J R & Deng L, *J Am Chem Soc*, 113, **1991**, 7063.
23 Suzumori K, Yasui Y, Asai H & Yagami Y, *Cancer Chemother*, 13, **1986**, 280.
24 Gent M, Knowlson P A & Rime F J, *Lancet*, 2, **1969**, 1094.
25 Egberts J, Fontijne P & Wamsteke K, *Biol Neonate*, 29, **1976**, 315.
26 Kidani Y, Inagaki K, Iigo M, Hoshi A & Kuretani K, *J Med Chem*, 21, **1978**, 1315.
27 Grange J M & Snell N J C, *J Ethnopharmacol*, 50, **1996**, 49.
28 Robin A, Monk H A, Wootton M, Walsh T R & Enright M C, *Lineages Emerginfec Dis*,10, **2004**, 855.
29 Vincent J L, Sakr Y, Sprung C L, Ranieri V M, Reinhart K, Gerlach H, Moreno R, Carlet J, Gall J L & Payen D, *Crit Care Med*, 34, **2006**, 344.
30 Capitano B, Leshem O A, Nightingale C H & Nicolau D P, *J Am Geriatr Soc*, 51, **2003**, 1.
31 Beena, Joshi S, Kumar N, Kidwai S, Singh R & Rawat D S, *Arch Pharm Chem Life Sci*, 345, **2012**, 896.
32 Sharma M, Joshi P, Kumar N, Joshi S, Rohilla R K, Roy M & Rawat D S, *Eur J Med Chem*, 46, **2011**, 480.
33 Sharma M, Roy N, Rohilla R K & Rawat D S, *Indian Patent Appl No*: 1462/DEL, **2008**.
34 Kumar D, Joshi S, Rohilla R K, Roy N & Rawat D S, *Bioorg Med Chem Lett*, 20, **2010**, 893.
35 Joshi M C, Bisht G S & Rawat D S, *Bioorg Med Chem Lett*, 17, **2007**, 3226.
36 Aggarwal N, Kumar R, Dureja P & Rawat D S, *J Agric Food Chem*, 57, **2009**, 8520.
37 Beena, Kumar N, Rohilla R K, Roy N & Rawat D S, *Bioorg Med Chem Lett*, 19, **2009**, 1396.
38 Bisht G S, Rawat D S, Kumar A, Kumar R & Pasha S, *Bioorg Med Chem Lett*, 17, **2007**, 4343.
39 Andrews J M, *J Antimicrob Chem*, 48, **2001**, 29.
40 Andrews J M, *J Antimicrob Chem*, 48, **2001**, 17.