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Synthesis and antituberculosis activity evaluation of cyclohexane-1,2-diamine derivatives

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Abstract: *Mycobacterium tuberculosis* is responsible for a large number of deaths worldwide. This bacterium is a matter of great concern as it has developed resistance against most of the anti-TB drugs available today. A series of new cyclohexane-1,2-diamine derivatives was synthesized and evaluated for their anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv *in vitro*. These compounds were found to be moderate to weakly active. Two compounds showed significant activity with minimum inhibitory concentrations of 11.8 and 13.1 µM (Optical Density).

Keywords: Tuberculosis, *M. tuberculosis*, cyclohexane-1,2-diamine, hydrochloride salt

Introduction

One of the largest public health concerns of the 21st century is tuberculosis (TB), caused by mycobacterium species such as *M. tuberculosis, M. avium* and *M. africanum*. It spreads *via* the respiratory route and mainly affects the lungs [1]. Although tuberculosis can be cured with a six months treatment regimen using the Directly Observed Treatment Short course (DOTS) [2], however the number of people infected with TB and the deaths reported every year is enormously high. According to World Health Organization (WHO), 9.4 million new TB cases and 1.7 million deaths were reported due to TB in 2010 [3]. About one-fifth of the global TB cases are reported in India alone [4]. The emergence of multidrug resistant (MDR), extensively drug resistant (XDR) and totally drug resistant (TDR) TB as well as convergence of TB with HIV has further worsened the situation [5-14]. Thus, there is an urgent need for new drugs for the treatment of drug resistant TB with novel mechanisms of action and short duration of treatment [15]. In recent years new drug candidates have been developed and some of which have also reached to early stages of clinical trials for TB. Presently, some new or repurposed drugs namely gatifloxacin, moxifloxacin, rifapentine, OPC67683, PA824, linezolid, PNU100480, AZD5847 and SQ109 are in different stages of clinical trials for the

treatment of tuberculosis [16-21].

The cyclohexyldiamine based compounds are known for their anti-cancer activity and recently we reported the antimicrobial activity of cyclohexane diamine based compounds Fig. (1) [22-27]. Structure activity relationship studies suggested that the introduction of an aliphatic chain in the benzene ring increases the antimicrobial activity of these compounds. To obtain more insights into the structural requirements and to study the complete activity pattern, new variations were incorporated in the aromatic nucleus. All the compounds (**11-14**) were evaluated for their *in vitro* anti-tubercular activity.

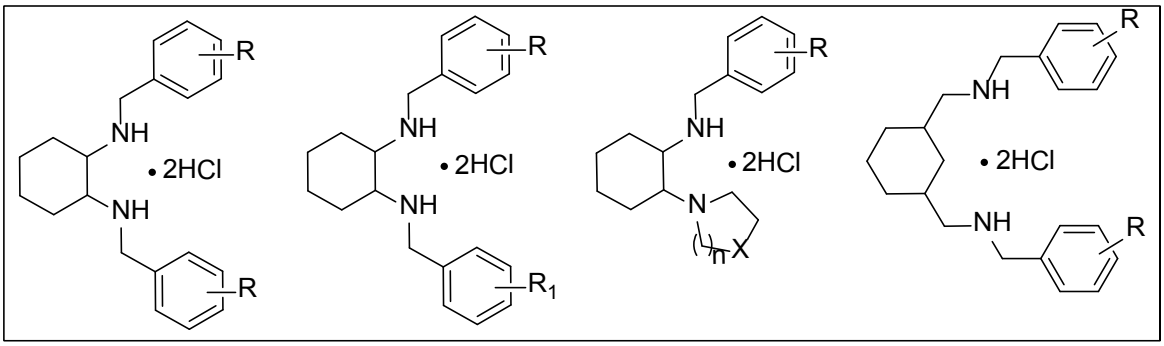


Figure 1: **Prototype structures of cyclohexane-diamine based antimicrobial agents**

Chemistry

To synthesize long chain containing cyclohexane-1,2-diamine derivatives, firstly aromatic aldehydes with different substituent were synthesized according to schemes 1, 2 and 3. The long chain acetylenic groups were introduced into the aromatic ring by the reaction of *ortho* or *para* bromobenzaldehyde (**1a**, **1b**) with terminal alkynes (**2a-2d**) using $[Pd(PPh_3)_2]Cl_2$ as a catalyst and the resulting compounds (**3a-3h**) were hydrogenated using hydrogen gas and Pd/C as a catalyst to get the desired long aliphatic chain containing benzaldehydes (**4a-4h**) as depicted in scheme 1 [28].

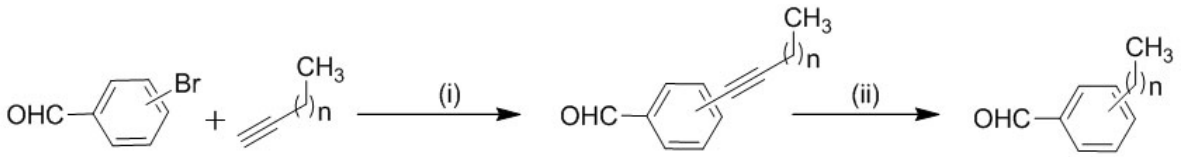
Benzaldehydes containing five or six membered heterocyclic rings at *para* position (**7a-7g**) were synthesized as shown in scheme 2. For this *para* fluorobenzaldehyde (**5**) was treated with nitrogen containing heterocycles (**6a-6g**) in the presence of K_2CO_3 at 80 °C using dimethylformamide as solvent [29]. The hydroxy benzaldehydes (**8a-8c**) were treated with alkyl halides in the presence of K_2CO_3 and DMF as solvent at room temperature, to get the desired substituted benzaldehydes (**9a-9i**) according to scheme 3 [30-36].

The benzaldehydes **3a-3h, 4a-4h, 7a-7g** and **9a-9i** synthesized under schemes 1, 2 and 3 were used for the preparation of cyclohexane-1,2-diamine derivatives. Cyclohexane-1,2-diamine (**10**) was treated with compounds **3a-3h, 4a-4h, 7a-7g** and **9a-9i** in dry MeOH at room temperature for 3-4 h to obtain imines which were reduced with $NaBH_4$ *in situ* to get the reduced product. These diamines were then converted to their hydrochloride salts by passing dry HCl gas to their solution in chloroform.

Biological Activity

In vitro antituberculosis activity

Minimum inhibitory concentration (MIC) was determined as the minimum compound concentration required to inhibit growth of *M. tuberculosis* in liquid medium completely.



1a, R = 2-Br
1b, R = 4-Br

2a, n = 2
2b, n = 3
2c, n = 4
2d, n = 5

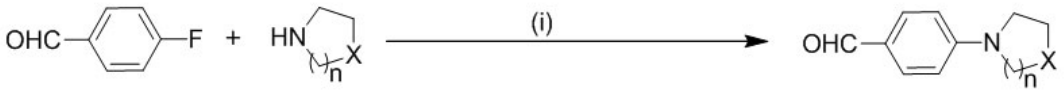
(3a-3h)

ortho: **3a**, n = 2
3b, n = 3
3c, n = 4
3d, n = 5
para: **3e**, n = 2
3f, n = 3
3g, n = 4
3h, n = 5

(4a-4h)

ortho: **4a**, n = 4
4b, n = 5
4c, n = 6
4d, n = 7
para: **4e**, n = 4
4f, n = 5
4g, n = 6
4h, n = 7

Scheme 1: Reagents and conditions:(i) Et_3N, $[Pd(PPh_3)]Cl_2$, CuI, Dry THF, 12 h, 50 ^oC, N_2 atm; (ii) H_2, 10% Pd/C, EtOAc, RT, 4-5 h



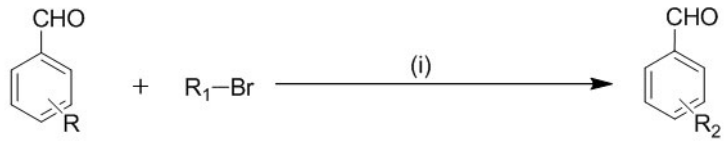
5

6a-6g

(7a-7g)

7a, n = 2, X = CH_2
7b, n = 2, X = NH
7c, n = 2, X = NHMe
7d, n = 2, X = NHEt
7e, n = 2, X = NHPh
7f, n = 2, X = O
7g, n = 1, X = CH_2

Scheme 2: Reagents and conditions: (i) K_2CO_3, Dry DMF, 80 ^oC, 8-10 h

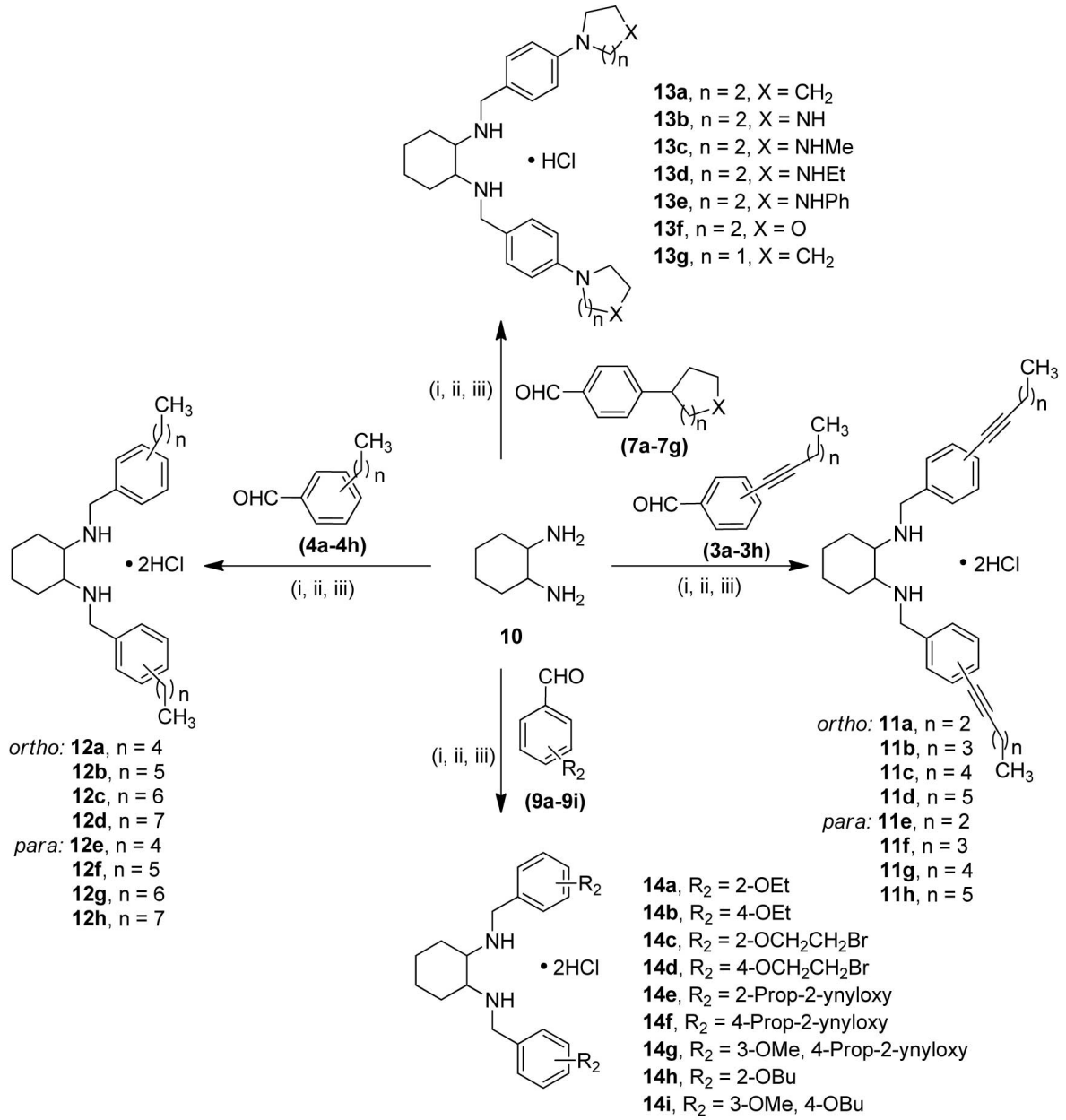


8a, R = 2-OH
8b, R = 4-OH
8c, R = 3-OMe, 4-OH

(9a-9i)

9a, R_2 = 2-OEt
9b, R_2 = 4-OEt
9c, R_2 = 2-OCH_2CH_2Br
9d, R_2 = 4-OCH_2CH_2Br
9e, R_2 = 2-Prop-2-ynyloxy
9f, R_2 = 4-Prop-2-ynyloxy
9g, R_2 = 3-OMe, 4-Prop-2-ynyloxy
9h, R_2 = 2-OBu
9i, R_2 = 3-OMe, 4-OBu

Scheme 3: Reagents and conditions: (i) K_2CO_3, Dry DMF, 8-10 h, RT



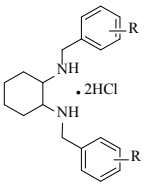
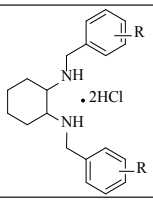
Scheme 4: Reagents and conditions: (i) Dry MeOH, RT, 3-4 h, (ii) $NaBH_4$, RT, 2-3 h (iii) $CHCl_3$, Dry HCl gas, 0.5 h

The assay was run in 96-well plates using a 10 point, two-fold serial dilution curve with the highest concentration of 20 μM. A fluorescent strain of *M. tuberculosis* was used - growth was measured over 5 days in the presence of compound in liquid medium (Middlebrook 7H9 broth, 10% oleic acid-albumen-dextrose-catalase supplement, 0.05% Tween 80). Two readouts of growth were used - optical density (OD) and fluorescence. MICs were calculated after curve fitting and are recorded for active compounds. Compounds with MIC >20 μM had >25% growth inhibition at the highest concentration; inactive compounds (NA) had <30% inhibition at the highest concentration.

Results and discussion

The thirty two cyclohexane-1,2-diamine derivatives (**11a-11h, 12a-12h, 13a-13g** and

Table 1: *In vitro* anti-tubercular activity of symmetrical cyclohexane-1,2-diamine derivatives (11-14)

Comp		MIC (µM)	MIC (µM)	Comp		MIC (µM)	MIC (µM)
	R	**OD**	**RFU**		**R**	**OD**	**RFU**
11a	2-Pent-1-ynyl	20.0	20.0	**13a**	4-(Piperidin-1-yl	>20.0	>20.0
11b	2-Hex-1-ynyl	>20.0	>20.0	**13b**	4-Piperizin-1-yl	NA	NA
11c	2-Hept-1-ynyl	>20.0	>20.0	**13c**	4-(4-Methylpiperazin-1-yl)	NA	NA
11d	2-Oct-1-ynyl	>20.0	>20.0	**13d**	4-(4-Ethylpiperazin-1-yl)	NA	NA
11e	4-Pent-1-ynyl	>20.0	>20.0	**13e**	4-(4-Phenylpiperazin-1-yl)	>20.0	NA
11f	4-Hex-1-ynyl	NA	NA	**13f**	4-Morpholino	NA	NA
11g	4-Hept-1-yny	>20.0	20.0	**13g**	4-Pyrrolyl	>20.0	>20.0
11h	4-Oct-1-ynyl	>20.0	>20.0	**14a**	2-OEt	>20.0	NA
12a	2-*n*-Pentyl	>20.0	>20.0	**14b**	4-OEt	NA	NA
12b	2-*n*-Hexyl	>20.0	>20.0	**14c**	2-OCH_2CH_2Br	20.0	20.0
12c	2-*n*-Heptyl	>20.0	>20.0	**14d**	4-OCH_2CH_2Br	>20.0	>20.0
12d	2-*n*-Octyl	>20.0	>20.0	**14e**	2- Prop-2-ynyloxy	NA	NA
12e	4-*n*-Pentyl	13.1	12.8	**14f**	4- Prop-2-ynyloxy	NA	NA
12f	4-*n*-Hexyl	11.8	14.1	**14g**	3-OMe, 4- prop-2-ynyloxy	NA	NA
12g	4-*n*-Heptyl	20.0	20.0	**14h**	2-OBu	NA	NA
12h	4-*n*-Octyl	>20.0	>20.0	**14i**	3-OMe, 4-OBu	>20.0	>20.0
EMB	2-3						

NA: Not active upto 20 µM

14a-14i) were tested for their anti-tubercular activity against the H37Rv strain of *M. tuberculosis* (table 1). Most of the compounds were found to be either weakly active or partially active with MIC ≥ 20 μM. Compounds **11a** and **12g** were active with MIC = 20 μM. Compounds **12e** and **12f** showed promising activity with MIC 13.1 and 11.8 μM, respectively. Compounds **11a**-**11h** with unsaturated chain at *ortho*/*para* position were weakly active. Amongst the compounds **12e**-**12h,** the five and six carbon saturated aliphatic chain compounds are active and with further increase in chain length (C-7 and C-8), the activity decreases.

Conclusion

In conclusion, a series of symmetrical cyclohexane-1,2-diamine derivatives were synthesized and screened for their *in vitro* anti-tubercular activity. Two compounds having 5 and 6 carbon chain at *para*-position of the benzene ring showed significant activity against *M. tb*. H37Rv. This observation is also consistent with our previous reports, which stated that on increasing the carbon chain length, anti-tubercular activity increases.

Experimental protocols

All the chemicals used in the syntheses were purchased from Sigma-Aldrich and were used as such. Thin layer chromatography (Merck TLC silica gel 60 F_{254}) 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 EZ-Melt automated melting point apparatus, Stanford Research Systems and are uncorrected. IR (KBr/nujol) spectra were recorded using Perkin-Elmer FT-IR spectrophotometer and the values are expressed as ν_{max} cm^{-1}. Mass spectral data were recorded in Jeol-AccuTOF JMS-T100LC. ^{1}H NMR spectra were recorded on Jeol ECX spectrospin 400 MHz instrument while ^{13}C NMR recorded at 100 MHz, using TMS as an internal standard. The chemical shift values were recorded on δ scale and the coupling constants (*J*) in Hz.

Synthesis of 4-(oct-1-ynyl)benzaldehyde (3h) and related compounds (3a-3h) [23]**:** To a mixture of 4-bromobenzaldehyde (1 g, 5.4 mmol), bis(triphenylphosphine)palladium(II) dichloride (189 mg, 0.270 mmol), 1-octyne (714 mg, 6.485 mmol) and Et_3N (1.093 g, 10.809 mmol) in dry THF (15 mL) cupper(I) iodide (102 mg, 0.540 mmol) was added. The mixture was stirred at 50 ^{o}C for 12 h under N_2 atmosphere (scheme 1). Insoluble materials (triethylammonium bromide etc.) were removed by filtration through a sintered funnel and solvent was removed. The crude product was dissolved in $CHCl_3$ and organic layer was washed with saturated brine (40 mL) and water (40 mL), dried under sodium sulphate and purified by silica gel column using hexane to afford pure compound **3h**. The compound was used immediately for the next reaction due to instability under air. Yield 81% (viscous pale yellow oil); IR (KBr, cm^{-1}): 2955, 2929, 2855, 2225, 1704; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (t, *J* = 7.3 Hz, 3H), 1.29-1.34 (m, 4H), 1.42-1.49 (m, 2H), 1.58-1.64 (m, 2H), 2.43 (t, *J* = 7.3 Hz, 2H), 7.52 (d, *J* = 8 Hz, 2H, Ar*H*), 7.79 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.98 (s, 1H, *CHO*).

Synthesis of 4-octyl-benzaldehyde (4h) and related compounds (4a-4h): Hydrogen gas was introduced into a vigorously stirred solution of compound **3h** (1 g) in deoxygenated ethyl acetate (20 mL) for 5 min *via* a syringe needle (scheme 1). To this solution a suspension of palladium/activated carbon (10% Pd, 60 mg) in ethyl acetate (5 mL) was added. The mixture was stirred for 4-5 h under H_2. After completion, reaction mixture was filtered through a sintered funnel and residue was thoroughly washed with hexane and excess solvent was removed under vacuum to yield dark yellow oil. Purification of the resultant oil by silica gel column chromatography using hexane as eluent yielded pure compound **4h**, which was used immediately

for the next reaction due to instability under air. Yield 83% (oily liquid); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87 (t, *J* = 7 Hz, 3H, CH_3), 1.26-1.31 (m, 10H), 1.58-1.67 (m, 2H), 2.68 (t, *J* = 8 Hz, 2H, CH_2), 7.33 (d, *J* = 8 Hz, 2H, Ar*H*), 7.79 (d, *J* = 8 Hz, 2H, Ar*H*), 9.97 (s, 1H, *CHO*).

Synthesis of *N*,*N*'-bis-(4-octyl-benzyl)-cyclohexane-1,2-diaminium dichloride (12h) and related compounds (11a-11h and **12a-12h):** To stirred solution of compound **4h** (600 mg, 2.748 mmol) in dry MeOH (15 mL) cyclohexane-1,2-diamine (**10**, 156 mg, 1.374 mmol) was added and the reaction mixture was stirred for 3-4 h at room temperature (scheme 4). To the reaction mixture sodium borohydride was added at 0 °C and stirring was continued for next 2-3 h at RT. After completion of the reaction, solvent was removed and the residue was extracted with $CHCl_3$. The organic layer was collected and dried over sodium sulphate, filtered and purified by column chromatography using MeOH-$CHCl_3$ as eluent. To the viscous liquid thus obtained, dry HCl gas was passed to obtain compound **12h**. Yield 80% (white solid); mp 180-182 °C; IR (film, cm^{-1}): 2926, 2855, 2737, 1589, 1458, 1217, 987; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87 (t, *J* = 7.3 Hz, 6H, 2 x CH_3), 1.24-1.28 (m, 18 H), 1.52 (brs, 4H), 1.65 (brs, 6H), 1.80 (brs, 2H), 1.94 (brs, 2H), 2.13-2.16 (m, 2H), 2.49-2.53 (m, 2H), 3.87 (brs, 2H), 3.91 (d, *J* = 12.4 Hz, 2H, CH_2Ph), 4.11 (d, *J* = 12.4 Hz, 2H, CH_2Ph), 7.15 (d, *J* = 8 Hz, 4H, Ar*H*), 7.55 (d, *J* = 8 Hz, 4H, Ar*H*), 9.49 (brs, 2H, NH_2^+), 10.47 (brs, 2H, NH_2^+); ESI-MS (*m/z*): 518.4 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(2-pent-1-ynyl-benzyl)-cyclohexane-1,2-diaminium dichloride (11a):** Yield 82% (white solid); mp 186-188 °C; IR (film, cm^{-1}): 2958, 2868, 2631, 2253, 1560, 1490, 1454, 1376, 1338, 1025, 1006; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90-0.94 (m, 6H, 2 x CH_3), 1.24-1.32 (m, 2H), 1.51 (sextet, *J* = 7 Hz, 4H, 2 x CH_3CH_2), 1.73-1.76 (m, 2H), 1.88 (brs, 3H), 2.05-2.06 (m, 1H), 2.29 (t, *J* = 7 Hz, 2H, $CH_3CH_2CH_2$), 2.35 (t, *J* = 7 Hz, 2H, $CH_3CH_2CH_2$), 3.76 (brs, 2H), 4.29 (brs, 2H, CH_2Ph), 4.37-4.46 (m, 2H, CH_2Ph), 7.15-7.24 (m, 3H, Ar*H*), 7.26-7.28 (m, 1H, Ar*H*), 7.31-7.34 (m, 2H, Ar*H*), 7.92-7.96 (m, 2H, Ar*H*), 9.72 (brs, 2H, NH_2^+), 10.70 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.33 (CH_3), 21.15 (CH_2), 21.21 (CH_2), 21.69 (CH_2), 22.81 (CH_2), 23.98 (CH_2), 26.57 (CH_2), 46.24 (CH_2), 47.62 (CH_2), 56.54 (*CH*), 57.21 (*CH*), 96.14 (PhC≡*C*), 96.81 (PhC≡*C*), 124.39 (*C*quart), 124.47(*C*quart), 128.02 (*CH*), 128.30 (*CH*), 128.93 (*CH*), 129.05 (*CH*), 130.96 (*CH*), 131.29 (*CH*), 131.37 (*CH*), 132.17 (*C*quart), 132.21 (*C*quart); ESI-MS (*m/z*): 426.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(2-hex-1-ynyl-benzyl)-cyclohexane-1,2-diaminium dichloride (11b):** Yield 88% (white solid); mp 180-182 °C; IR (film, cm^{-1}): 2932, 2860, 2709, 2629, 1552, 1457, 1377, 1322, 1248, 995, 863, 792; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (t, *J* = 7 Hz, 6H, 2 x CH_3), 1.41-1.47 (m, 6H), 1.56 (sextet, *J* = 7 Hz, 4H, 2 x CH3$CH_2CH_2CH_2$), 1.81 (brs, 4H), 1.91 (brs, 1H), 2.07 (brs, 1H), 2.34 (t, *J* = 7Hz, 2H, $CH_3CH_2CH_2CH_2$), 2.41 (t, *J* = 7 Hz, 2H, $CH_3CH_2CH_2CH_2$), 3.72 (brs, 1H), 3.80 (brs, 1H), 4.17 (d, *J* = 12.4 Hz, 1H, CH_2Ph), 4.32 (d, *J* = 12.4 Hz, 1H, CH_2Ph), 4.47-4.52 (m, 2H, CH_2Ph), 7.23-7.27 (m, 2H, Ar*H*), 7.29-7.32 (m, 2H, Ar*H*), 7.37-7.42 (m, 2H, Ar*H*), 7.94 (d, *J* = 7.3 Hz, 1H, Ar*H*), 8.03 (d, *J* = 7.3 Hz, 1H, Ar*H*), 9.14 (brs, 1H, NH_2^+), 10.04 (brs, 1H, NH_2^+), 10.32 (brs, 1H, NH_2^+), 11.19 (brs, 1H, NH_2^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.29 (CH_3), 17.77 (CH_2), 20.57 (CH_2), 21.56 (CH_2), 25.30 (CH_2), 29.13 (CH_2), 44.77 (CH_2), 46.47 (CH_2), 55.54 (*CH*), 76.33 (*CH*), 95.27 (PhC≡*C*), 95.53 (PhC≡*C*), 123.29 (*C*quart), 123.46 (*C*quart), 126.84 (Ar-*CH*), 127.93 (Ar-*CH*), 129.82 (Ar-*CH*), 130.48 (Ar-*CH*), 131.11 (*C*quart); ESI-MS (*m/z*): 454.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(2-hept-1-ynyl-benzyl)-cyclohexane-1,2-diaminium dichloride (11c):** Yield 75% (white solid); mp 172-174 °C; IR

(KBr, cm^{-1}): 2932, 2860, 2729, 1550, 1463, 994; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.92 (t, *J* = 7 Hz, 6H, 2 x CH_3), 1.30-1.43 (m, 10H), 1.55-1.62 (m, 4H), 1.79 (brs, 2H), 1.89 (brs, 2H), 2.32-2.36 (m, 2H), 2.40 (t, *J* = 7.3 Hz, 4H, 2 x $CH_3CH_2CH_2CH_2CH_2$), 3.80 (brs, 2H), 4.16 (d, *J* = 13.2 Hz, 2H, CH_2Ph), 4.31 (d, *J* = 13.2 Hz, 2H, CH_2Ph), 7.24-7.33 (m, 4H, Ar*H*), 7.39-7.41 (m, 2H, Ar*H*), 7.94 (d, *J* = 7.3 Hz, 2H, Ar*H*), 10.07 (brs, 2H, NH_2^+), 10.33 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.94 (CH_3), 19.34 (CH_2), 19.55 (CH_2), 22.17 (CH_2), 23.08 (CH_2), 27.24 (CH_2), 28.30 (CH_2), 31.20 (CH_2), 45.62 (CH_2), 56.66 (*CH*), 77.67 (Ph*C*≡C), 97.03 (PhC≡*C*), 124.53 (*C*quart), 128.25 (Ar-*CH*), 129.05 (Ar-*CH*), 130.99 (Ar-*CH*), 131.55 (Ar-*CH*), 132.47 (*C*quart); ESI-MS (*m/z*): 482.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(2-oct-1-ynyl-benzyl)-cyclohexane-1,2-diaminium dichloride (11d):** Yield 86% (white solid); mp 162-164 °C; IR (KBr, cm^{-1}): 2931, 1458, 999, 845; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.90 (t, *J* = 7 Hz, 6H, 2 x CH_3), 1.29-1.33 (m, 8H), 1.39-1.46 (m, 4H), 1.56-1.61 (m, 4H), 1.74 (brs, 4H), 1.88 (brs, 1H), 1.96 (brs, 1H), 2.04-2.07 (m, 1H), 2.19-2.22 (m, 1H), 2.38 (t, *J* = 7 Hz, 4H, 2 x $CH_3CH_2CH_2CH_2CH_2CH_2$), 3.31 (brs, 2H), 3.76 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 3.99 (d, *J* = 11 Hz, 2H, CH_2Ph), 4.14 (d, *J* = 12.5 Hz, 1H, CH_2Ph), 7.33 (d, *J* = 8 Hz, 3H, Ar*H*), 7.38 (d, *J* = 8 Hz, 1H, Ar*H*), 7.62 (d, *J* = 8 Hz, 4H, Ar*H*), 8.90 (brs, 2H, NH_2^+), 10.24 (brs, 2H, NH_2^+); ESI-MS (*m/z*): 510.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(4-pent-1-ynyl-benzyl)-cyclohexane-1,2-diaminium dichloride (11e):** Yield 90% (white solid); mp 154-156 °C; IR (film, cm^{-1}): 2932, 2859, 2709, 1512, 1457, 1376, 1212, 985; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.04 (t, *J* = 7.3 Hz, 6H, 2 x CH_3), 1.32 (brs, 2H), 1.60-1.65 (m, 4H), 1.88 (brs, 2H), 2.09-2.17 (m, 4H), 2.38 (t, *J* = 7.3 Hz, 4H, 2 x $CH_3CH_2CH_2$), 3.15 (brs, 2H), 3.67 (brs, 2H, CH_2Ph), 3.92 (brs, 2H, CH_2Ph), 7.30 (d, *J* = 6.5 Hz, 4H, Ar*H*), 7.62 (s, 4H, Ar*H*), 8.84 (brs, 2H, NH_2^+), 10.23 (brs, 2H, NH_2^+); ESI-MS (*m/z*): 426.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(4-hex-1-ynyl-benzyl)-cyclohexane-1,2-diaminium dichloride (11f):** Yield 92% (white solid); mp 154-156 °C; IR (KBr, cm^{-1}): 2521, 2363, 2261, 1458, 1194, 884, 796, 644; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.92 (t, *J* = 7 Hz, 6H, 2 x CH_3), 1.34-1.43 (m, 8H), 1.56-1.63 (m, 4H), 1.67 (brs, 1H), 1.87 (brs, 1H), 2.04-2.06 (m, 1H), 2.16-2.19 (m, 1H), 2.39 (t, *J* = 7Hz, 4H, 2 x $CH_3CH_2CH_2CH_2$), 3.27 (brs, 2H), 3.73 (d, *J* = 11.7 Hz, 2H, CH_2Ph), 3.97 (d, *J* = 11.7 Hz, 2H, CH_2Ph), 7.33 (d, *J* = 8 Hz, 3H, Ar*H*), 7.38 (d, *J* = 8 Hz, 1H, Ar*H*), 7.62 (d, *J* = 8 Hz, 4H, Ar*H*), 8.88 (brs, 2H, NH_2^+), 10.28 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.86 (CH_3), 18.69 (CH_2), 21.81 (CH_2), 22.60 (CH_2), 26.07 (CH_2), 30.53 (CH_2), 47.74 (CH_2), 56.87 (*CH*), 80.37 (Ph*C*≡C), 92.35 (PhC≡*C*), 124.44 (*C*quart), 130.86 (Ar-*CH*), 131.38 (Ar-*CH*), 131.77 (*C*quart); ESI-MS (*m/z*): 454.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(4-hept-1-ynyl-benzyl)-cyclohexane-1,2-diaminium dichloride (11g):** Yield 74% (white solid); mp 180-182 °C; IR (KBr, cm^{-1}): 2932, 2860, 2729, 1550, 1463, 994, 760; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.92 (t, *J* = 7 Hz, 6 H, 2 x CH_3), 1.31-1.35 (m, 10H), 1.56-1.63 (m, 4H), 1.66-1.67 (m, 1H), 1.87 (brs, 2H), 2.04-2.06 (m, 1H), 2.16-2.22 (m, 2H), 2.39 (t, *J* = 6.6 Hz, 4H, 2 x $CH_3CH_2CH_2CH_2CH_2$), 3.27 (brs, 2H), 3.72 (d, *J* = 11.7 Hz, 2H, CH_2Ph), 3.96 (d, *J* = 11.7 Hz, 2H, CH_2Ph), 7.33 (d, *J* = 8 Hz, 4H, Ar*H*), 7.62 (d, *J* = 8 Hz, 4H, Ar*H*), 8.88 (brs, 2H, NH_2^+), 10.28 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.98 (CH_3), 19.34 (CH_2), 22.16 (CH_2), 22.87 (CH_2), 26.46 (CH_2), 28.36 (CH_2), 31.10 (CH_2), 48.70 (CH_2), 58.06 (*CH*), 79.80 (Ph*C*≡C), 92.13 (PhC≡*C*), 125.14 (*C*quart), 129.70 (Ar-*CH*), 131.41 (Ar-*CH*), 131.53 (Ar-*CH*), 131.93 (Cquart); ESI-MS (*m/z*): 482.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(4-oct-1-ynyl-benzyl)-cyclohexane-1,2-diaminium dichloride (11h):** Yield 65%

(white solid); mp 180-182 °C; IR (film, cm^{-1}): 2929, 2857, 2730, 1458, 1212, 1112; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, *J* = 7.3 Hz, 6H, 2 x CH_3), 1.29-1.33 (m, 8H), 1.39-1.46 (m, 4H), 1.54-1.60 (m, 6H), 1.87-1.89 (m, 2H), 2.02-2.04 (m, 2H), 2.19 (d, *J* = 10.9 Hz, 2H), 2.39 (t, *J* = 7.3 Hz, 4H, 2 x $CH_3CH_2CH_2CH_2CH_2CH_2$), 3.33 (brs, 2H), 3.77 (d, *J* = 11.7 Hz, 2H, CH_2Ph), 4.0 (d, *J* = 11.7 Hz, 2H, CH_2Ph), 7.33-7.39 (m, 4H, Ar*H*), 7.61 (d, *J* = 8 Hz, 4H, Ar*H*), 8.93 (brs, 2H, NH_2^+), 10.32 (brs, 2H, NH_2^+); ESI-MS (*m/z*): 510.3 $[M - 2HCl]^+$.

***N*,*N*'-Bis-(2-pentyl-benzyl)-cyclohexane-1,2-diaminium dichloride (12a):** Yield 82% (white solid); mp 186-188°C; IR (film, cm^{-1}): 2925, 2856, 2626, 2256, 1656, 1454, 1366, 1024; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.88 (t, *J* = 6.6 Hz, 6H, 2 x CH_3), 1.30-1.36 (m, 9H), 1.39 (brs, 1H), 1.52-1.53 (m, 5H), 1.88-1.90 (m, 3H), 1.99 (brs, 1H), 2.20-2.23 (m, 1H), 2.66-2.75 (m, 4H), 3.80 (brs, 2H), 3.99 (d, *J* = 11 Hz, 2H, CH_2Ph), 4.11 (d, *J* = 11Hz, 2H, CH_2Ph), 7.15-7.29 (m, 6H, Ar*H*), 7.77-7.82 (m, 2H, Ar*H*), 9.32 (brs, 2H, NH_2^+), 10.33 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.24 (CH_3), 21.69 (CH_2), 22.30 (CH_2), 26.02 (CH_2), 30.09 (CH_2), 30.68 (CH_2), 30.73 (CH_2), 32.10 (CH_2), 44.98 (CH_2), 46.17 (CH_2), 57.04 (*CH*), 57.60 (*CH*), 125.65 (Ar-*CH*), 128.01(Ar-*CH*), 128.62 (Ar-*CH*), 128.84 (Ar-*CH*), 128.93 (Ar-*CH*), 130.59 (Ar-*CH*), 141.25 (*C*quart), 141.44 (*C*quart); ESI-MS (*m/z*): 434.3 $[M - 2HCl]^+$.

***N*,*N*'-Bis-(2-hexyl-benzyl)-cyclohexane-1,2-diaminium dichloride (12b):** Yield 74% (white solid); mp 208-210 °C; IR (KBr, cm^{-1}): 2929, 2857, 2709, 2633, 1458, 1000; ^1H NMR (400 MHz, DMSO-d_6) δ: 0.84 (brs, 8H), 1.27 (brs, 16H), 1.48-1.50 (m, 4H), 1.78 (brs, 2H), 2.66-2.68 (m, 4H), 3.68 (brs, 1H), 3.86 (brs, 1H), 4.19 (brs, 2H, CH_2Ph), 4.29-4.31 (m, 2H, CH_2Ph), 7.24-7.25 (m, 4H, Ar*H*), 7.29-7.31 (m, 2H, Ar*H*), 7.68-7.70 (m, 2H, Ar*H*), 9.56 (brs, 2H, NH_2^+), 9.68 (brs, 2H, NH_2^+); ESI-MS (*m/z*): 462.3 $[M - 2HCl]^+$.

***N*,*N*'-Bis-(2-heptyl-benzyl)-cyclohexane-1,2-diaminium dichloride (12c):** Yield 82% (white solid); mp 196-198 °C; IR (film, cm^{-1}): 2926, 2853, 2624, 1651, 1452, 1025; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.89 (m, 6H, 2 x CH_3), 1.26-1.29 (m, 18H), 1.61 (brs, 1H), 1.72 (brs, 4H), 1.89-1.92 (m, 3H), 2.03 (brs, 1H), 2.17 (brs, 1H), 2.67 (brs, 4H), 3.80 (brs, 2H), 3.99-4.11 (m, 4H, 2CH_2Ph), 7.13-7.18 (m, 3H, Ar*H*), 7.23-7.25 (m, 3H, Ar*H*), 7.79-7.83 (m, 2H, Ar*H*), 9.29 (brs, 1H, NH_2^+), 9.49 (brs, 1H, NH_2^+), 10.06 (brs, 1H, NH_2^+), 10.28 (brs, 1H, NH_2^+); ESI-MS (*m/z*): 490.4 $[M - 2HCl]^+$.

***N*,*N*'-Bis-(2-octyl-benzyl)-cyclohexane-1,2-diaminium dichloride (12d):** Yield 60%; mp 144-146 °C (white solid); IR (film, cm^{-1}): 2926, 2854, 2767, 1593, 1458, 1217, 1121; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.87 (t, *J* = 6.6 Hz, 6H, 2 x CH_3), 1.24-1.28 (m, 24H), 1.79-1.80 (m, 4H), 1.91 (brs, 2H), 2.13 (brs, 1H), 2.16 (brs, 1H), 2.51 (t, *J* = 7.7 Hz, 4H), 3.76 (brs, 2H), 3.90 (d, *J* = 12 Hz, 2H, CH_2Ph), 4.09 (d, *J* = 12 Hz, 2H, CH_2Ph), 7.15 (d, *J* = 7.3 Hz, 4H, Ar*H*), 7.54 (d, *J* = 8 Hz, 4H, Ar*H*), 9.96 (brs, 4H, 2NH_2^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.08 (CH_3), 22.63 (CH_2), 23.09 (CH_2), 27.35 (CH_2), 29.22 (CH_2), 29.36 (CH_2), 29.40 (CH_2), 31.20 (CH_2), 31.83 (CH_2), 35.67 (CH_2), 48.53 (CH_2), 57.33 (*CH*), 127.19 (Ar-*CH*), 128.96 (Ar-*CH*), 130.70 (Ar-*CH*), 144.51 (*C*quart); ESI-MS (*m/z*): 518.4 $[M - 2HCl]^+$.

***N*,*N*'-Bis-(4-pentyl-benzyl)-cyclohexane-1,2-diaminium dichloride (12e):** Yield 75% (white solid); mp 140-142 °C; IR (KBr, cm^{-1}): 2928, 2856, 2709, 1452, 1194, 829; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86 (t, *J* = 7 Hz, 6H, 2 x CH_3), 1.21-1.34 (m, 10H), 1.52 (quintet, *J* = 7 Hz, 4H), 1.78-1.80 (m, 2H), 1.94 (brs, 2H), 2.10 (brs, 1H), 2.13 (brs, 1H), 2.51 (t, *J* = 7 Hz, 4H, 2 x $CH_3CH_2CH_2CH_2CH_2$), 3.75 (brs, 2H), 3.86 (d, *J* = 12.4 Hz, 2H, CH_2Ph), 4.06 (d, *J* = 12.4 Hz, 2H, CH_2Ph), 7.14 (d, *J* = 8 Hz, 4H, Ar*H*), 7.56 (d, *J* = 8 Hz, 4H, Ar*H*), 9.41 (brs, 2H, NH_2^+), 10.42 (brs, 2H, NH_2); ESI-MS (*m/z*): 434.3 [M

- 2HCl]$^+$.

***N,N'*-Bis-(4-hexyl-benzyl)-cyclohexane-1,2-diaminium dichloride (12f):** Yield 78% (white solid); mp 124-126 ^{o}C; IR (film, cm^{-1}): 2928, 2857, 2258, 2124, 1647, 1420, 1137, 1025, 995; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.84-0.88 (m, 6H), 1.25-1.29 (m, 14H), 1.45-1.53 (m, 4H), 1.77 (brs, 2H), 1.93 (brs, 2H), 2.11-2.14 (m, 2H), 2.50-2.56 (m, 4H), 3.74 (brs, 2H), 3.90 (d, *J* = 12 Hz, 2H, CH_2Ph), 4.09 (d, *J* = 12 Hz, 2H, CH_2Ph), 7.14 (d, *J* = 7.8 Hz, 4H, Ar*H*), 7.56 (d, *J* = 7.8 Hz, 4H, Ar*H*), 9.90-10.53 (m, 4H, NH_2^+); ESI-MS (*m/z*): 462.3 [M - 2HCl]$^+$.

***N,N'*-Bis-(4-heptyl-benzyl)-cyclohexane-1,2-diaminium dichloride (12g):** Yield 80% (white solid); mp 182-184 ^{o}C; IR (film, cm^{-1}): 2926, 2855, 2734, 2217, 1913, 1582, 1517, 1455, 1377, 1310, 1217, 1189, 1121, 1048, 985, 921; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (t, *J* = 6.6 Hz, 6H, 2 x CH_3), 1.23-1.26 (m, 19H), 1.50-1.53 (m, 2H), 1.78-1.80 (m, 3H), 1.97 (brs, 2H), 2.10-2.13 (m, 2H), 2.51 (t, *J* = 8 Hz, 4H, 2 x $CH_3CH_2CH_2CH_2CH_2CH_2CH_2$), 3.76 (brs, 2H), 3.87 (d, *J* = 12.4 Hz, 2H, CH_2Ph), 4.06 (d, *J* = 12.4 Hz, 2H, CH_2Ph), 7.14 (d, *J* = 8 Hz, 4H, Ar*H*), 7.56 (d, *J* = 8 Hz, 4H, Ar*H*), 9.41 (brs, 2H, NH_2^+), 10.44 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.01 (CH_3), 22.55 (CH_2), 22.81 (CH_2), 27.03 (CH_2), 29.05 (CH_2), 29.26 (CH_2), 31.13 (CH_2), 31.70 (CH_2), 35.63 (CH_2), 48.50 (CH_2), 57.22 (*CH*), 127.12 (Ar-*CH*), 128.75 (Ar-*CH*), 130.87 (*C*quart), 144.26 (*C*quart); ESI-MS (*m/z*): 491.51 [M - 2HCl]$^+$.

Synthesis of 4-(piperidin-1-yl)benzaldehyde (7a) and related compounds (7b-7g) [24]

To a well stirred solution of piperidine (2 g, 0.023 mol) in dry DMF (20 mL), anhydrous K_2CO_3 (3.17 g, 0.023 mol) was added and stirred for 15 min. To this mixture, *p*-fluorobenzadehyde (1 g, 7.66 mmol) was added and reaction mixture was stirred at 80 ^{o}C for 10 h (scheme 2). After completion of the reaction water was added to the reaction mixture and extracted with $CHCl_3$. The organic layer was dried over sodium sulphate, filtered and solvent was removed. The crude product thus obtained was purified by column chromatography.

Synthesis of *N,N'*-bis(4-(piperidin-1-yl)benzyl)cyclohexane-1,2-diaminium tetrachloride (13a) and related compounds (13b-13g)

To a stirred solution of compound **7a** (500 mg, 2.64 mmol) in dry MeOH (15 mL) cyclohexane-1,2-diamine (**10**, 150 mg, 1.32 mmol) was added and the reaction mixture was stirred for 3-4 h at room temperature (scheme 4). To the reaction mixture sodium borohydride was added at 0 ^{o}C and stirring was continued for next 2-3 h at RT. After completion of the reaction, solvent was removed and the residue was extracted with $CHCl_3$. The organic layer was collected and dried over sodium sulphate, filtered and purified by column chromatography using MeOH-$CHCl_3$ as eluent. To the viscous liquid thus obtained, dry HCl gas was passed to obtain compound **13a**. Yield 67% (white solid); mp 198-199 ^{o}C; IR (KBr, cm^{-1}): 2947, 2866, 2653, 2524, 1578, 1518, 1458, 1260, 1198, 1158, 1012, 951; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.21 (br, 2H), 1.61 (brs, 4H), 1.63-1.69 (m, 2H), 1.73-1.75 (m, 4H), 1.83 (brs, 6H), 2.29 (d, *J* = 10.9 Hz, 2H), 3.36 (s, 8H, 4 x CH_2NH^+), 3.50 (brs, 2H, 2 x *CH*), 4.14 (brs, 2H, CH_2Ph), 4.27 (brs, 2H, CH_2Ph), 7.69 (s, 8H, Ar*H*), 9.72 (brs, 2H, NH_2^+), 9.86 (brs, 2H, NH_2^+); ESI-MS (*m/z*): 460.4 [M - 4HCl]$^+$

***N,N'*-Bis(4-(piperazin-1-yl)benzyl) cyclohexane-1,2-diaminium hexachloride (13b):** Yield 76% (yellow solid); mp 62-64 ^{o}C; IR (KBr, cm^{-1}): 2930, 2778, 2717, 1611, 1520, 1451, 1256, 1040, 925; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.18-1.22 (m, 2H), 1.38 (brs, 1H), 1.64-1.82 (m, 3H), 2.27-2.29 (m, 2H), 3.17 (s, 8H), 3.40 (s, 8H, 4 x $CH_2NH_2^+$), 3.65 (brs, 1H, *CH*), 3.85-3.89 (m, 1H, *CH*), 4.07 (brs, 2H,

CH_2Ph), 4.15-4.19 (m, 2H, CH_2Ph), 4.24 (brs, 2H, 2 x NH^+), 7.0-7.05 (m, 4H, Ar*H*), 7.51 (d, *J* = 8.7 Hz, 4H, Ar*H*), 9.35 (brs, 4H, 2 x NH_2^+), 9.74 (brs, 4H, 2 x NH_2^+); ESI-MS (*m/z*): 462.3 $[M - 6HCl]^+$.

***N,N'*-Bis(4-(4-methylpiperazin-1-yl)benzyl) cyclohexane-1,2-diaminium hexachloride (13c):** Yield 61% (hygroscopic brown solid); IR (KBr, cm^{-1}): 2935, 1617, 1450, 1240, 907; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.14-1.21 (m, 2H), 1.41-1.47 (m, 2H), 1.62 (brs, 2H), 1.70 (brs, 2H), 2.13 (d, *J* = 10 Hz, 1H), 2.23 (d, *J* = 10 Hz, 1H), 2.45 (s, 6H, 2 x CH_3NH^+), 3.07-3.10 (m, 8H, 4 x CH_2), 3.39-3.41 (m, 8H, 4 x CH_2), 3.99-4.02 (m, 2H, CH_2Ph), 4.11-4.17 (m, 2H, CH_2Ph), 6.98 (d, *J* = 8.4 Hz, 4H, Ar*H*), 7.48 (d, *J* = 8.4 Hz, 4H, Ar*H*), 8.74 (brs, 2H, 2 x NH^+), 9.76 (brs, 2H, NH_2^+), 9.83 (brs, 2H, NH_2^+), 11.28 (brs, 2H, 2 x NH^+); ESI-MS (*m/z*): 490.3 $[M - 6HCl]^+$.

***N,N'*-Bis(4-(4-ethylpiperazin-1-yl)benzyl) cyclohexane-1,2-diaminium hexachloride (13d):** Yield 78% (pale yellow hygroscopic solid); IR (KBr, cm^{-1}): 2949, 2863, 2491, 2373, 1619, 1457, 1263, 1117, 1011; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.17 (brs, 2H), 1.28 (t, *J* = 7.3 Hz, 6H, 2 x CH_2CH_3), 1.65-1.71 (m, 4H), 2.27 (d, *J* = 10.9 Hz, 2H), 3.03-3.08 (m, 4H), 3.13-3.19 (m, 8H), 3.41 (brs, 2H), 3.50 (d, *J* = 10.9 Hz, 4H), 3.84-3.93 (m, 8H), 4.14 (brs, 2H), 7.02 (d, *J* = 8 Hz, 4H, Ar*H*), 7.51 (d, *J* = 8 Hz, 4H, Ar*H*), 9.80 (brs, 4H, 2 x NH_2^+), 11.26 (brs, 2H, 2 x NH^+); ESI-MS (*m/z*): 518.4 $[M - 6HCl]^+$.

***N,N'*-Bis(4-(4-phenylpiperazin-1-yl)benzyl) cyclohexane-1,2-diamine hexachloride (13e):** Yield 78% (pale yellow solid); mp 155-157 °C; IR (KBr, cm^{-1}): 2944, 2863, 2491, 2373, 1619, 1457, 1263, 1117, 1011; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.20-1.21 (m, 2H), 1.67 (brs, 2H), 1.74 (brs, 1H), 1.84 (brs, 1H), 2.30 (d, *J* = 12.4 Hz, 2H), 3.44 (s, 2H, 2 X CH), 3.56-3.58 (m, 16H, 4 x NCH_2CH_2N), 4.18 (brs, 4H, 2 x CH_2Ph), 7.16 (d, *J* = 7.7 Hz, 6H, Ar*H*), 7.39-7.43 (m, 4H, Ar*H*), 7.48 (brs, 4H, Ar*H*), 7.56 (d, *J* = 8.7 Hz, 4H, Ar*H*), 8.92-8.94 (m, 2H, 2 x NH^+), 9.52 (brs, 2H, 2 x NH^+), 9.76-9.81 (m, 4H, 2NH_2^+); ESI-MS (*m/z*): 614.4 $[M - 6HCl]^+$.

***N,N'*-Bis(4-morpholinobenzyl)cyclohexane-1,2-diaminium tetrachloride (13f):** Yield 75% (off white solid); mp 182-184 °C; IR (KBr, cm^{-1}): 2990, 2970, 2872, 2711, 2345, 1574, 1519, 1459, 1259, 1128, 1068, 1040; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.15-1.20 (m, 2H), 1.62-1.89 (m, 4H), 2.28 (d, *J* = 11.4 Hz, 2H), 3.14-3.16 (m, 8H, 4 x CH_2), 3.41 (brs, 2H, 2 x *CH*), 3.73-3.76 (m, 8H, 4 x CH_2), 3.98-4.14 (m, 4H, 2 x CH_2Ph), 7.06 (d, *J* = 8.2 Hz, 4H, Ar*H*), 7.51 (d, *J* = 8.2 Hz, 4H, Ar*H*), 9.43 (brs, 2H, 2 x NH^+), 9.73 (brs, 4H, 2 x NH_2^+); ESI-MS (*m/z*): 464.3 $[M - 4HCl]^+$.

***N,N'*-Bis(4-(pyrrolidin-1-yl)benzyl) cyclohexane-1,2-diaminium tetrachloride (13g)**: Yield 60%; mp 183-184 °C; IR (KBr, cm^{-1}): 2957, 2865, 2711, 2637, 2375, 1459, 1189, 1028, 902; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.14-1.19 (m, 2H), 1.55-1.58 (m, 2H), 1.68 (brs, 2H), 1.91 (s, 8H, 2 x CH_2CH_2), 2.24 (d, *J* = 10.9 Hz, 2H), 3.19 (s, 8H, 4 x CH_2), 3.32 (brs, 2H, 2 x *CH*), 3.97-4.05 (m, 4H, 2 x CH_2Ph), 6.56 (d, *J* = 8 Hz, 4H, Ar*H*), 7.38 (d, *J* = 8 Hz, 4H, Ar*H*), 9.58 (brs, 4H, 2 x NH_2^+), 9.64 (brs, 2H, 2 x NH^+).

Synthesis of 2-Butoxybenzaldehyde (9h) and related compounds (9a-9i) [25-31]

To a solution of *o*-hydroxy benzaldehyde (1 g, 8.18 mol) in dry DMF (15 mL), anhydrous K_2CO_3 (24.5 mol, 3.4 g) was added and stirred for 15 min. To this reaction mixture *n*-butyl bromide (1.12 g, 8.18 mol) was added and stirred at room temperature for 8-10 h (scheme 1.8). After completion of the reaction, water was added to the reaction mixture and extracted with $CHCl_3$. The organic layer was dried over sodium sulphate, filtered and solvent removed.

The crude product was purified by column chromatograpy to get compound **9h**.

Synthesis of *N*,*N*'-bis-(2-butoxy-benzyl)-cyclohexane-1,2-diaminium dichloride (14h) and related compounds (14a-14i)

To stirred solution of compound **9h** (600 mg, 2.748 mmol) in dry MeOH (15 mL) cyclohexane-1,2-diamine (**10**, 156 mg, 1.374 mmol) was added and the reaction mixture was stirred at room temperature (scheme 4). After 3-4 h sodium borohydride was added to it at 0 ^{o}C and stirred for next 2-3 h at RT. After completion of the reaction, solvent was removed and the residue was extracted with $CHCl_3$. The organic layer was collected and dried over sodium sulphate, filtered and purified by column chromatography using MeOH-$CHCl_3$ as eluent. To the viscous liquid thus obtained, dry HCl gas was passed to obtain compound **14h**. Yield 88% (white solid); mp 170-172 ^{o}C; IR (KBr, cm^{-1}): 2955, 2871, 2753, 2642, 1605, 1559, 1497, 1457, 1252, 1125; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.91 (t, *J* = 7.3 Hz, 6H, 2 x CH_3), 1.27 (brs, 2H), 1.39-1.47 (m, 4H, $OCH_2CH_2CH_2CH_3$), 1.71-1.72 (m, 7H), 1.84-1.96 (m, 1H), 2.34 (d, *J* = 11.9 Hz, 2H), 3.46 (brs, 1H, *CH*), 3.70 (brs, 1H, *CH*), 4.01 (t, *J* = 6.4 Hz, 4H, 2 x OCH_2), 4.13 (brs, 4H, 2 x CH_2Ph), 6.94-6.98 (m, 2H, Ar*H*), 7.06 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.36-7.39 (m, 2H, Ar*H*), 7.59 (d, *J* = 6.8 Hz, 2H, Ar*H*), 9.53 (brs, 2H, NH_2^+), 9.76 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.79 (CH_3), 18.78 (CH_2), 22.29 (CH_2), 25.84 (CH_2), 30.66 (CH_2), 42.37 (CH_2), 55.88 (*CH*), 67.61 (OCH_2), 111.71 (*C*quart), 119.08 (Ar-*CH*), 120.13 (Ar-*CH*), 130.98 (Ar-*CH*), 132.30 (Ar-*CH*), 157.10 (*C*quart); ESI-MS (*m/z*): 438.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(2-ethoxy-benzyl)-cyclohexane-1,2-diaminium dichloride (14a):** Yield 91% (white solid); mp 212-214 ^{o}C; IR (KBr, cm^{-1}): 2945, 2868, 2763, 1648, 1604, 1560, 1498, 1458, 1293, 1256, 1123, 1045; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.24-1.27 (m, 2H), 1.33 (t, *J* = 6.8 Hz, 6H, 2 x OCH_2CH_3), 1.74 (brs, 4H), 2.33-2.35 (m, 2H), 3.51 (brs, 2H, 2 x *CH*), 4.03-4.09 (m, 4H, 2 x OCH_2), 4.10 (brs, 4H, 2 x CH_2Ph), 6.94 (t, *J* = 7 Hz, 2H, Ar*H*), 7.03 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.34-7.38 (m, 2H, Ar*H*), 7.57 (d, *J* = 7 Hz, 2H, Ar*H*), 9.70-9.75 (m, 4H, 2 x NH_2^+); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 15.12 (CH_3), 22.78 (CH_2), 26.66 (CH_2), 44.16 (CH_2), 57.19 (*CH*), 64.47 (OCH_2), 112.49 (Ar-*CH*), 119.65 (Ar-*CH*), 121.04 (*C*quart), 132.12 (Ar-*CH*), 132.71 (Ar-*CH*), 157.67 (*C*quart); ESI-MS (*m/z*): 382.2 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(4-ethoxy-benzyl)-cyclohexane-1,2-diaminium dichloride (14b):** Yield 90% (white solid); mp 202-204 ^{o}C; IR (KBr, cm^{-1}): 2632, 1634, 1519, 1251, 1190, 1045; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.13-1.18 (m, 2H), 1.29 (t, *J* = 7 Hz, 6H, 2 x OCH_2CH_3), 1.70 (br, 4H), 2.24 (d, *J* = 11.9 Hz, 2H), 3.47 (brs, 2H, 2 x *CH*), 4.0 (q, *J* = 7 Hz, 4H, 2 x OCH_2CH_3), 4.06 (brs, 2H, CH_2Ph), 4.14-4.16 (m, 2H, CH_2Ph), 6.92 (d, *J* = 8.7 Hz, 4H, Ar*H*), 7.55 (d, *J* = 8.7 Hz, 4H, Ar*H*), 9.80 (brs, 2H, NH_2^+), 10.03 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.59 (CH_3), 22.20 (CH_2), 25.61 (CH_2), 46.98 (CH_2), 55.60 (*CH*), 63.09 (OCH_2), 114.31(Ar-*CH*), 123.01 (*C*quart), 132.04 (Ar-*CH*), 158.93 (*C*quart); ESI-MS (*m/z*): 382.2 [M - 2HCl]$^+$.

***N*,*N*'-Bis(2-(2-bromoethoxy)benzyl) cyclohexane-1,2-diaminium dichloride (14c):** Yield 86% (white solid); mp 88-90 ^{o}C; IR (KBr, cm^{-1}): 2943, 2871, 2726, 1603, 1495, 1455, 1293, 1246, 1196, 1121, 1053, 938; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.74-0.95 (m, 1H), 1.08 (brs, 1H), 1.16-1.21 (m, 2H), 1.50-1.81 (m, 3H), 2.16 (brs, 1H), 3.55-3.71 (m, 2H), 4.05-4.23 (m, 4H, 2CH_2Ph), 4.41 (s, 6H), 4.65-4.77 (m, 2H), 6.93-7.0 (m, 2H, Ar*H*), 7.05-7.11 (m, 1H, Ar*H*), 7.14-7.17 (m, 1H, Ar*H*), 7.31-7.33 (m, 1H, Ar*H*), 7.38-7.40 (m, 2H, Ar*H*), 7.49-7.56 (m, 1H, Ar*H*), 8.82 (brs, 1H, NH_2^+), 9.36 (brs, 1H, NH_2^+), 9.85-9.94 (m, 2H, NH_2^+); ESI-MS (*m/z*): 538.1 [M - 2HCl]$^+$, 540.2 [M+2 - 2HCl]$^+$

***N*,*N*'-Bis-[4-(2-bromo-ethoxy)-benzyl]-cyclohexane-1,2-diaminium dichloride (14d):** Yield 84%; mp 172-174 °C; IR (KBr, cm^{-1}): 2933, 2863, 2711, 2633, 1610, 1516, 1458, 1249, 1183, 1073, 1016; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.18-1.21 (m, 2H), 1.65 (brs, 2H), 1.72 (brs, 2H), 2.29 (d, *J* = 11.4 Hz, 2H), 3.43 (brs, 2H), 3.79 (t, *J* = 5.5 Hz, 4H, 2 x CH_2Br), 4.09 (d, *J* = 12.4 Hz, 2H, CH_2Ph), 4.20-4.27 (m, 2H, CH_2Ph), 4.32 (t, *J* = 5.5 Hz, 4H, 2 x OCH_2), 6.99 (d, *J* = 8.7 Hz, 4H, Ar*H*), 7.56 (d, *J* = 8.7 Hz, 4H, Ar*H*), 9.74 (brs, 4H, 2NH_2^+); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.11 (CH_2), 25.53 (CH_2), 31.51 (CH_2Br), 47.07 (CH_2), 55.74 (*CH*), 67.87 (OCH_2), 68.05 (OCH_2), 114.63 (Ar-*CH*), 123.84 (*C*quart), 132.11 (Ar-*CH*), 158.42 (*C*quart); ESI-MS (*m/z*): 538.0 [M - 2HCl]$^+$, 540.2 [M+2 - 2HCl]$^+$.

***N*,*N*'-Bis-(2-prop-2-ynyloxy-benzyl)-cyclohexane-1,2-diaminium dichloride (14e):** Yield 89% (white solid); mp 144-146 °C; IR (KBr, cm^{-1}): 3207, 2941, 2867, 2769, 2705, 2623, 2552, 2500, 2460, 2383, 1605, 1589, 1492, 1463, 1297, 1260, 1226, 1191, 1123, 1020; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.23 (brs, 2H), 1.72 (brs, 4H), 2.32 (d, *J* = 10.5 Hz, 2H), 3.47 (brs, 2H), 3.63-3.64 (m, 2H), 4.11 (s, 4H), 4.81-4.90 (m, 4H), 6.98-7.02 (m, 2H, Ar*H*), 7.13 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.37-7.41 (m, 2H, Ar*H*), 7.59 (d, *J* = 6.8 Hz, 2H, Ar*H*), 9.70 (brs, 2H, NH_2^+), 9.92 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.28 (CH_2), 25.79 (CH_2), 45.51 (CH_2), 56.01 (*CH*), 56.16 (*CH*), 78.77 (C≡*CH*), 79.03 (*C*≡CH), 112.46 (Ar-*CH*), 119.62 (Ar-*CH*), 121.14 (*C*quart), 130.91 (Ar-*CH*), 132.30 (Ar-*CH*), 158.85 (*C*quart); ESI-MS (*m/z*): 402.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(4-prop-2-ynyloxy-benzyl)-cyclohexane-1,2-diaminium dichloride (14f):** Yield 85% (white solid); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.19-1.21 (m, 1H), 1.36 (brs, 1H), 1.72 (brs, 2H), 1.83 (brs, 2H), 2.28 (d, *J* = 11.7 Hz, 1H), 3.49 (brs, 2H, 2 x *CH*), 3.56 (t, *J* = 2.2 Hz, 2H, 2 x C≡*CH*), 3.70 (brs, 1H), 4.10 (brs, 2H, CH_2Ph), 4.21 (brs, 2H, CH_2Ph), 4.80 (d, *J* = 2.2 Hz, 4H, OCH_2), 7.01 (dd, *J* = 8.8, 3.6 Hz, 4H, Ar*H*), 7.59 (dd, *J* = 8.8, 3.6 Hz, 4H, Ar*H*), 9.56 (brs, 1H, NH_2^+), 9.72 (brs, 1H, NH_2^+), 9.89 (brs, 2H, NH_2^+); ESI-MS (*m/z*): 402.3 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(3-methoxy-4-prop-2-ynyloxy-benzyl)-cyclohexane-1,2-diaminium dichloride (14g):** Yield 90% (white solid); mp 210-212 °C; IR (KBr, cm^{-1}): 2957, 2866, 2706, 2631, 1518, 1466, 1458, 1271, 1226, 1151, 1015; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.17 (brs, 1H), 1.38 (brs, 1H), 1.64-1.89 (m, 4H), 2.28-2.30 (m, 2H), 3.34 (brs, 2H, 2 x *CH*), 3.52-3.54 (m, 2H, 2 x C≡*CH*), 3.77 (s, 6H, 2 x OCH_3), 4.07-4.09 (m, 2H, CH_2Ph), 4.21 (brs, 2H, CH_2Ph), 4.77-4.79 (m, 4H, 2 x OCH_2), 7.00-7.03 (m, 2H, Ar*H*), 7.08-7.11 (m, 2H, Ar*H*), 7.45-7.47 (m, 2H, Ar*H*), 9.55-9.97 (m, 4H, 2NH_2^+); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.25 (CH_2), 25.67 (CH_2), 47.43 (CH_2), 48.75 (CH_2), 55.70 (OCH_3), 55.80 (*CH*), 55.95 (OCH_2), 78.42 (*C*≡CH), 79.17 (C≡*C*H), 113.60 (Ar-*CH*), 114.45 (Ar-*CH*), 122.68 (Ar-*CH*), 122.83 (Ar-*CH*), 124.56 (*C*quart), 146.87 (*C*quart), 149.03 (*C*quart); ESI-MS (*m/z*): 462.2 [M - 2HCl]$^+$.

***N*,*N*'-Bis-(4-butoxy-3-methoxy-benzyl)-cyclohexane-1,2-diaminium dichloride (14i):** Yield 82% (white solid); mp 154-156 °C; IR (KBr, cm^{-1}): 3066, 2955, 2871, 2712, 2630, 1624, 1518, 1466, 1427, 1263, 1239, 1170, 1145, 1064, 1029; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.90 (t, *J* = 7.3 Hz, 6H, 2 x OCH$_2$CH$_2$CH$_2$*CH*$_3$), 1.14 (brs, 2H), 1.39 (sextet, *J* = 7.3 Hz, 4H, 2 x OCH$_2$CH$_2$*CH*$_2$CH$_3$), 1.64-1.69 (m, 8H), 2.25 (d, *J* = 10.5 Hz, 2H), 3.44 (brs, 2H), 3.76 (s, 6H, 2 x OCH_3), 3.92 (t, *J* = 6.4 Hz, 4H, 2 x OCH_2), 4.05 (d, *J* = 12.3 Hz, 2H, CH_2Ph), 4.17 (d, *J* = 12.3 Hz, 2H, CH_2Ph), 6.92 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.06 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.46 (s, 2H, Ar*H*), 9.90 (brs, 2H, NH_2^+), 10.17 (brs, 2H, NH_2^+); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.75 (CH_3), 18.81 (CH_2), 22.27 (CH_2), 25.68 (CH_2), 30.81 (CH_2), 47.42 (CH_2), 55.51 (*CH*), 55.70 (OCH_3),

67.84(OCH_2), 112.46 (Ar-*CH*), 114.26 (Ar-*CH*), 123.0 (Ar-*CH*), 123.32 (*C*quart), 148.61 (*C*quart), 148.82 (*C*quart); ESI-MS (*m/z*): 498.3 $[M - 2HCl]^+$.

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