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Original article

Synthesis, antimicrobial activity and structure–activity relationship study of *N*,*N*-dibenzyl-cyclohexane-1,2-diamine derivatives

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ABSTRACT

We report herein synthesis and antimicrobial activity of a series of *N*,*N*-dibenzyl-cyclohexane-1,2-diamine derivatives. In order to study the structure–activity relationship of substituted dibenzyl-cyclohexane-1,2-diamine derivatives, 44 structurally diverse compounds were synthesized and tested against Gram-positive and Gram-negative bacterial strains. Among them, compounds **17-20**, **26**, **37**, **38** were found to be more active than tetracycline with MIC value ranging 0.0005–0.032 μg/mL and no hemolysis upto 1024 μg/mL in mammalian erythrocytes was observed. Some of the compounds have also shown very promising antifungal activity against *Candida albicans*, *Candida glabrata* and *Geotrichum candidium*.

1. Introduction

Bacterial infections have been most deleterious to the human health and cause more deaths than HIV infections [1–4]. The multi-drug resistance both in the community and hospitals has been the major concerns to public health and scientific community world-wide [5–8]. The incidence of bacterial resistance has been observed to different class of antibacterials such as β-lactams, macrolides, quinolones, glycopeptides, oxazolidinones etc. and by 2003 over 59% resistant isolates have been registered [9]. Among the resistant strains, an infection caused by methicillin resistance *Staphylococcus aureus* (MRSA) and drug resistant enterococci is difficult to treat and as of now vancomycin is the last defense against these infections [10–13]. Despite the fact that incidence of multi-drug resistance is continuously increasing, pharma industry has decreased its research on the development of new antibacterials, which is evident by the fact that in last 40 years only two antibiotics namely, linezolid and daptomycin have been introduced in the market [14–16]. In order to improve quality of life there is an urgent need for the development of new chemical entities that can solve the problem of drug resistance.

To achieve this goal, different approaches such as identification of novel targets where no pre-existing resistance exist and exploration of existing clinically proven targets for new chemical entities are being explored [17]. Unfortunately, identification of novel drug targets have not been successful due to issues associated with target validation and low hit rates from high throughput screening but the second approach has been used successfully by scientists across the globe to identify new chemical entities as antibacterial agents [18].

As a part of our on going programme towards the development of new class of antimicrobial agents [19–24], we decided to functionalize bromhexine (**1a**, benzyl-cyclohexyl derivative) which is used for the treatment of respiratory disorders [25,26]. Antituberculosis activities of bromhexine derivatives are known but antibacterial potential of this class of compounds have not been fully exploited [27]. Other closely related compound **1b** that can be considered as dimer of bromhexine derivatives is based on 1, 2-diaminocyclohexanes (DACHs), which have been widely used as a carrier ligand in the development of *cis*-platin based anticancer molecules [28–31]. Ormaplatin [32] and oxaliplatin [33] are analogues of *cis*-platin which are now in clinical trials that bears DACH as carrier ligand. To the best of our knowledge antimicrobial activity of these compounds have not been reported. The medicinal

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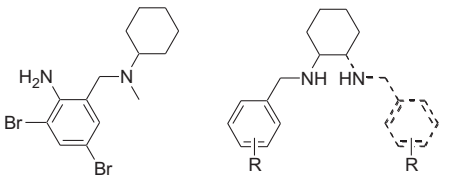
potential of bromhexine and DACH derivatives, and the fact that this scaffold offers great opportunity to generate library of simple compounds, prompted us to modify bromhexine by functionalizing 1,2-diaminocyclohexane and study their antimicrobial activities. To this end we report herein synthesis and antimicrobial activity of *N*,*N*-dibenzyl-cyclohexane-1,2-diamine based compounds.



Bromhexine (1a) **Partial dimer of modified bromhexine derivatives (1b)**

2. Chemistry

Synthesis of the title compounds was accomplished as outlined in Scheme 1, using one or two step synthetic protocols reported elsewhere [34]. Two-step procedures involve the reaction of 2 mol of substituted benzaldehydes and 1 mol of diamine in dry methanol which leads to the formation of desired imines (**3**–**12**) [35–40]. The isolated imines were reduced to amines by the use of sodium borohydride, and all the compounds were characterized by standard analytical and spectroscopic methods. In one step procedure imines was reduced in-situ using sodium borohydride as reducing agent.

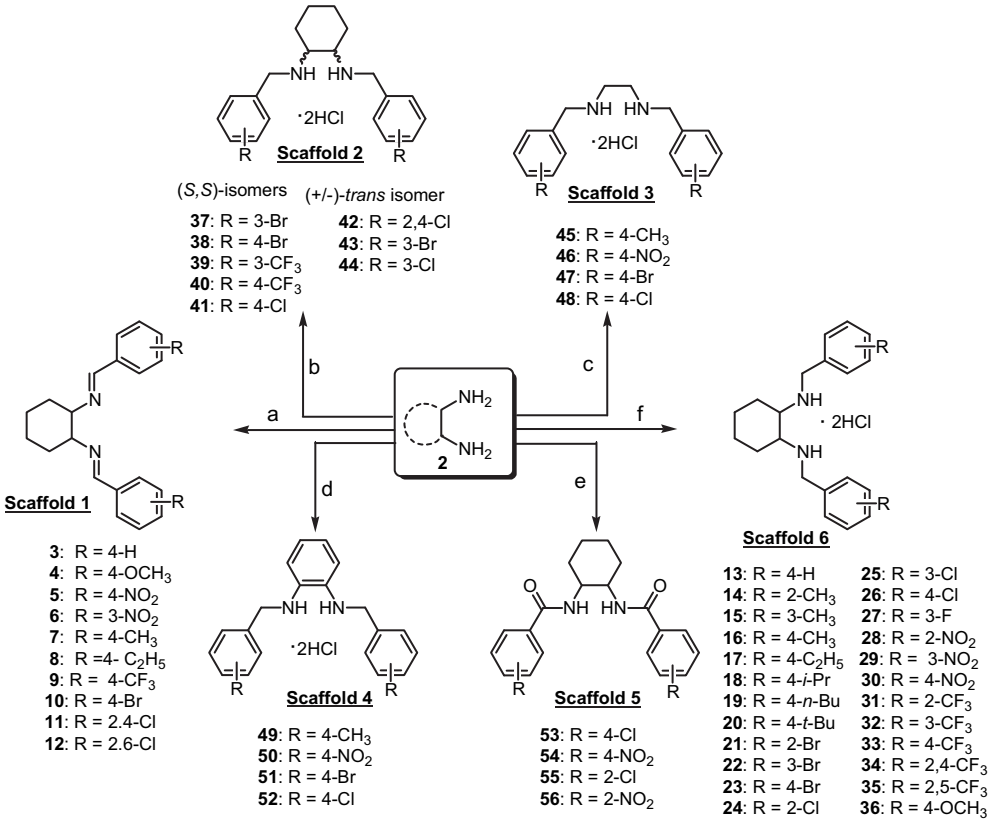
3. Biological activity

3.1. In vitro antibacterial activity

Antimicrobial susceptibility testing was carried out using National Committee for Clinical Laboratory Standards (NCCLS) micro dilution assay. Briefly, the bacterial strains were grown in standard media until exponential growth was achieved. Tests were performed in the 96-well microtiter plate in a final volume of 100 μl. Test compounds were dissolved in 5% DMSO at an initial concentration of 500 μg/mL and serially diluted in plate. Each well was then inoculated with ~2–5 × 10^5 bacterial cells and incubated at 37 °C for 24 h with shaking at 200 rpm. One well containing bacterial cells (~2–5 × 10^5) and 5% DMSO without any test compound (growth control), and one well containing only growth medium (sterility control) were used as controls. Growth of bacteria was determined using Power wave200 microplate scanning spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA). Percent survival was calculated using growth without any compound as 100% survival. The MIC values were calculated using Grafit 4.0 software (Erithacus Software Ltd., Horley, Surrey, UK).

3.2. In vitro antifungal activity

Some of the compounds were also tested on *Geotrichum candidum* NCIM 980, *Candida albicans* MTCC 3017 and *Candida glabrata* MTCC 3019. Tests were performed in a 96-well microtiter plate in a final volume of 100 μl. Test compounds were dissolved in 5% DMSO at an initial concentration of 500 μM and serially diluted in plate (500–3.9 μg/mL) using MGYP media (0.3% malt extract, 1%



Scheme 1. Reagents: a) 1,2-diaminocyclohexane, benzaldehydes, mol. sieves; b) (*S*,*S*), and (±)-trans-1,2-diaminocyclohexane, benzaldehydes, mol. sieves, $NaBH_4$, HCl gas; c) 1,2-diaminoethane, benzaldehydes, mol. sieves, $NaBH_4$, HCl gas; d) *o*-phenylenedamine, benzaldehydes, mol. sieves, $NaBH_4$, HCl gas; e) benzoic acid, ethylchloroformate, triethylamine, 1,2-diaminocyclohexane; f) 1,2-diaminocyclohexane, benzaldehydes, mol. sieves, $NaBH_4$, HCl gas.

glucose, 0.3% yeast extract, and 0.5% peptone). Each well was then inoculated with ~2–5 × 10^5 fungal cells and incubated at 30 °C for 24 h with shaking at 200 rpm. One well containing fungal cells (~2–5 × 10^5) 5% DMSO without any test compound (growth control) and one well containing only growth medium (sterility control) were used as controls. Growth of fungi was determined using Power wave200 microplate scanning spectrophotometer (Bio-Tek InstrumentsÒ, Winooski, VT, USA). Percent survival was calculated using growth without any compound as 100% survival.

4. Results and discussion

The minimum inhibitory concentrations (MIC) of the compounds (**13**–**44**) against Gram-negative strains (*E. coli* and *P. aeruginosa*) and Gram-positive strains (*S. aureus* and *S. epidermidis*) are shown in Table 1 along with tetracycline which was used as a standard antibiotic in this study. Most of the compounds were found to be active against both Gram-negative and Gram-positive bacterial strains. Structure–activity relationship study revealed that alkyl substitution at the phenyl ring improves antibacterial activity (entry **14**–**20**). Among these compounds a clear trend of improved activity was noticed in the order of methyl, ethyl, *iso*-propyl, *n*-butyl and *tert*-butyl substitution in the phenyl ring (**14**–**20**). Interestingly Schiff base counter parts of these compounds were found to be totally inactive even at higher concentration. Substitution of bromo, chloro, or nitro groups at the *ortho* position of the phenyl ring had negative affect on the antibacterial activity (entry **21**, **24**, **28**). In order to understand the role of stereochemistry on the antibacterial activity of these compounds, some compounds were prepared by using stereochemically pure 1,2-diaminocyclohexane and (±)-*trans*-1,2-diaminocyclohexane as a starting material and a marginal effect was observed. For example, stereochemically pure isomer **38** in which the bromo group is at the *para* position of the phenyl ring shows potent antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* with MIC values 0.008, 0.008, 0.002 and 0.016 ug/mL respectively, while racemic compound **23** exhibit activity against same strains with MIC values 0.25, 0.032, 0.016 and 0.032 ug/mL respectively. Same trend was observed in other compounds as well (**22** *vs* **37**, **26** *vs* **41**, **32** *vs* **39**, **33** *vs* **40**). In order to understand the role of basicity of nitrogen, amide analogues of **24**, **26**, **28**, **30** were prepared and all the amides (**53**–**56**) were found to be inactive even at higher concentration. All the Schiff bases (**3**–**12**) and amides (**53**–**56**) were found to be totally inactive which suggest that free rotation around the C–N bond is essential for the antibacterial activity of these compounds. To investigate the role of cyclohexane backbone on antibacterial activity, another series of compounds having 1,2-diamino-ethylene (**45**–**48**) and 1,2-diamino-phenyl (**49**–**52**) linker were synthesized, and all these compounds were found to be inactive even at very high concentration. Thus with the change in cyclohexane backbone and basicity of amines we arrived at the conclusion that free rotation around the C–N bond along with the presence of cyclohexane backbone is essential for the antibacterial activity of these compounds (Fig. 1).

We observed that lipophilicity may play an important role in antibacterial activity of these compounds because different substituents on benzene ring significantly alter the Clog *P* value (R = 4-NO_2, Clog P = 4.67 and R = 4-*n*-Bu, Clog P = 9.35). To rationalize this result we obtained log *P* (Clog *P*) which represents a measure of lipophilicity and correlated it with MIC value of amines (**13**–**44**) Table 1. It showed that progressive increase in lipophilicity of the compounds acquired by replacing the substituent R on phenyl ring with nitro groups (**28**–**30**), OMe (**36**), H (**13**), halogens [F (**27**) < Cl (**24**–**26**, **41**, **44**) < Br (**21**–**23**, **37**, **43**)], methyl

Table 1
Antibacterial activity (MIC) of *N*,*N*-dibenzyl-cyclohexane-1,2-diamine derivatives (μg/mL).

Comp No	R	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. epidermidis*	Clog *P*
13	4-H	Not active	Not active	Not active	Not active	5.18
14	2-Me	0.125	0.125	0.25	0.25	6.18
15	3-Me	0.125	0.065	0.032	0.25	6.18
16	4Me	0.25	0.125	0.125	0.25	6.18
17	4-Et	0.008	0.004	0.008	0.008	7.24
18	4-*i*-Pr	0.004	0.0005	0.008	0.008	8.03
19	4-*n*-Bu	0.008	0.0005	0.008	0.008	9.35
20	4-*t*-Bu	0.008	0.0006	0.004	0.008	8.83
21	2-Br	Not active	Not active	Not active	Not active	
22	3-Br	0.065	0.25	0.008	0.065	6.91
23	4-Br	0.25	0.032	0.016	0.032	6.91
24	2-Cl	Not active	Not active	Not active	Not active	
25	3-Cl	0.032	0.008	0.032	0.032	6.61
26	4-Cl	0.008	0.008	0.008	0.008	6.61
27	3-F	0.25	0.25	Not active	0.25	5.47
28	2-NO_2	Not active	Not active	Not active	Not active	4.67
29	3-NO_2	0.5	0.25	0.5	1.0	4.67
30	4-NO_2	Not active	0.125	Not active	Not active	4.67
31	2-CF_3	0.25	0.032	0.065	0.065	6.95
32	3-CF_3	0.065	0.25	0.016	0.032	6.95
33	4-CF_3	0.032	0.008	0.008	0.032	6.95
34	2,4-CF_3	Not active	Not active	Not active	Not active	
35	2,5-CF_3	Not active	Not active	Not active	Not active	
36	4-OCH_3	Not active	Not active	Not active	Not active	
37	3-Br (*S*,*S*)	0.032	0.008	0.008	0.032	6.91
38	4-Br (*S*,*S*)	0.008	0.008	0.002	0.016	6.91
39	3-CF_3 (*S*,*S*)	0.032	0.008	0.016	0.065	6.5
40	4-CF_3 (*S*,*S*)	0.032	0.008	0.016	0.032	6.95
41	4-Cl	0.032	0.032	0.032	0.032	6.91
42	2,4-Cl (±)	0.032	0.016	0.016	0.032	8.04
43	3-Br (±)	0.016	0.032	0.008	0.032	6.91
44	3-Cl (±)	0.032	0.065	Not active	0.032	6.91
	Tetracycline	0.001	0.001	0.001	0.002	

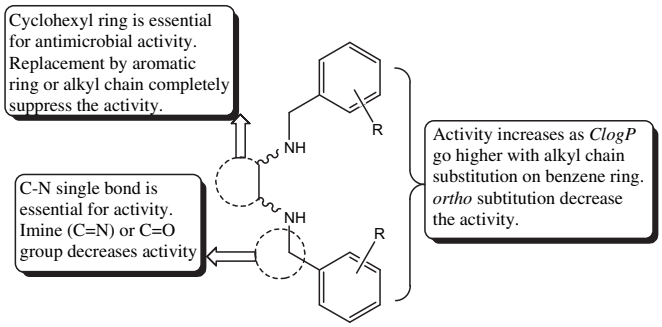


Fig. 1. SAR study of N,N-dibenzyl-cyclohexane-1,2-diamine derivatives

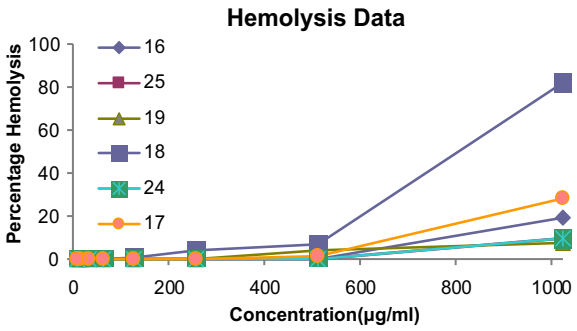


Fig. 2. Hemolytic activity of the compounds. Fresh hRBC suspension (4% v/v in 35 mM phosphate buffer with 150 mM NaCl) was used for the assay. After incubation of the test sample in the erythrocyte solution for 1 h at 37 °C, the solution was centrifuged and the supernatant absorbance was determined at 414 nm. Hemolysis affected by 0.1% Triton X-100 was considered as 100%.

(**14**–**16**), trifluoromethyl (**31**–**33**, **39**, **40**) and alkyl chain (**14**–**20**) respectively, had same effect on MIC values. There is one exception in pattern of lipophilicity with compounds **34** and **35** those have higher Clog *P* value and found to be inactive.

Some of the compounds were also tested for their antifungal activity against *C. albicans, C. glabrata* and *G. candidium* and showed potent antifungal activity with MIC ranging from 0.0005–0.125 μg/mL (Table 2). Interestingly all the best active antibacterial compounds (17–20) have also shown potent antifungal activity and these compounds were found to be 37–6200 fold more active than reference compound. All the tested compounds have shown potent activity against *G. candidium*.

Toxicity of these compounds were investigated using human red blood cells (hRBC) and results showed that compounds **14**, **20**–**23**, **26**–**38** and **40** did not show any toxicity even at the highest concentration tested of 1024 μg/mL concentration, while compounds **15**, **16**, **17**, **18**, **19**, **24**, **25**, **39**, **41** showed 4.78, 19.19, 38.33, 81.81, 7.68, 9.83, 9.68, 3.70, and 4.80% hemolysis respectively at 1024 μg/mL concentration (Fig. 2).

5. Conclusions

We synthesized a series *N,N*-dibenzyl-cyclohexane-1,2-diamine derivatives, and evaluated them for their antibacterial and antifungal activity. Some of the compounds have shown better antibacterial and antifungal activity than the reference compounds. A number of compounds designed and synthesized in the study were found to be 37–6200 fold more active than reference compound against *C. albicans*, *C. Glabrata* and *G. candidum*. All the compounds found to be non-toxic against mammalian erythrocytes. Further derivatization, *in vivo* activity and oral acute toxicity evaluation of these compounds is under progress and results will be published in due course of time.

Table 2
Antifungal activity (MIC) of *N,N*-dibenzyl-cyclohexane-1,2-diamine derivatives (μg/mL).

Comp No	R	*C. albicans*	*C. glabrata*	*G. candidum*
14	2-Me	Not active	Not active	0.25
15	3-Me	Not active	Not active	0.25
16	4-Me	Not active	Not active	0.25
17	4-Et	0.032	0.032	0.032
18	4-*i*-Pr	0.008	0.016	Not active
19	4-*n*-Bu	0.008	0.008	0.008
20	4-*t*-Bu	0.008	0.008	0.008
22	3-Br	0.125	0. 25	0.065
23	4-Br	0.125	0.125	0.032
25	3-Cl	0.125	0.125	0.065
26	4-Cl	0.125	0.125	0.032
27	3-F	Not active	Not active	0.5
30	4-NO_2	Not active	Not active	0.5
31	2-CF_3	0.5	Not active	0.5
32	3-CF_3	0.125	0.25	0.125
33	4-CF_3	0.125	0.125	0.065
37	3-Br (*S,S*)	0.125	0.065	0.065
38	4-Br (*S,S*)	0.065	0.032	0.032
39	3-CF_3 (*S,S*)	Not active	0.125	0.125
40	4-CF_3 (*S,S*)	0.125	0.065	0.065
44	3-Cl (±)	0.125	Not active	0.032
Amphotericin		0.30	50.0	6.25

6. Experimental protocols

All of the chemicals used in the syntheses were purchased from Sigma-Aldrich and were used as such. Thin layer chromatography was used to monitor the progress of the reactions. The compounds were purified by silica gel column. Melting points were determined on a melting point apparatus and are uncorrected. IR (KBr) spectra were recorded using Perkin-Elmer FT-IR spectrophotometer and the values are expressed as υ_{max} cm^{-1}. Mass spectral data were recorded in waters micromass LCT Mass Spectrometer/Data system. The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Spectrospin spectrometer at 300 MHz, 400 MHz and 75.5 MHz, 100 MHz respectively using TMS as an internal standard. The chemical shift values are recorded on δ scale and the coupling constants (*J*) are in Hz. Elemental analysis were performed on a Carlo Erba Model EA-1108 elemental analyzer and data of C, H and N is within ±0.4% of calculated values.

*6.1. General procedure of the synthesis of substituted diamines: synthesis of N,N′-bis-(4-tert-butyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**20**)*

To a solution of 4-*tert*-butyl-benzaldehyde (500 mg, 3.08 mmol) in dry tetrahydrofuran (5 mL), 1,2-diaminocyclohexane (175 mg, 1.54 mmol) was added. The reaction mixture was stirred for 3–4 h and progress of reaction was monitored by thin layer chromatography (Scheme 1). To this reaction mixture sodium borohydride (141 mg, 3.72 mmol) was added and stirred for 3 h at room temperature (Scheme 1). Reaction was quenched by the addition of cold water and extracted with chloroform (2 × 15 mL). Organic layer was dried over anhydrous sodium sulphate and excess of solvent was removed under reduced pressure to afford pale yellow oil. In a 100 mL, three-necked flask equipped with magnetic stirrer, dry HCl gas was passed to the solution of this oil in chloroform (20 mL), and white solid of **20** was precipitated out which was filtered and washed with chloroform (20 mL).

*6.1.1. N,N′-dibenzyl-cyclohexane-1,2-diaminiumchloride (**13**)*

Yield: 92%; Mp: 240 °C; IR (KBr, cm^{-1}): 2946, 2715, 2641, 1624, 1458, 988, 754, 698, 508; ^{1}H NMR (300 MHz, DMSO-d_6): 10.23–9.93 (m, 4H), 7.69–7.38 (m, 10H), 4.27 (d, J = 12 Hz, 2H), 4.12 (d, J = 12 Hz, 2H), 3.75–3.58 (s, 2H), 2.30–2.27 (m, 2H), 1.85–1.75 (m, 4H), 1.18 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 132.38, 131.28, 129.74, 129.36, 56.92, 48.40, 26.54, 23.10; ESI-HRMS calculated for $C_{20}H_{28}Cl_2N_2$: 366.1630, Found: 294.2095 [M − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{28}Cl_2N_2$: C, 65.39; H, 7.68; N, 7.63; found: C, 65.40; H, 7.42; N, 7.84.

*6.1.2. N,N′-Bis-(2-methyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**14**)*

Yield: 90%; Mp: 219 °C; IR (KBr, cm^{-1}): 2942, 2865, 2717, 1646, 1560, 1464, 1319, 1038, 788, 753; ^{1}H NMR (300 MHz, DMSO-d_6): 10.13 (brs, 2H), 9.79 (brs, 2H), 7.69–7.67 (m, 2H), 7.30–7.19 (m, 6H), 4.29–4.19 (m, 4H), 3.81 (s, 2H), 2.43–2.41 (m, 8H), 1.27 (s, 2H), 1.80 (s, 4H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 138.50, 132.02, 131.24, 131.03, 129.82, 126.72, 57.88, 46.13, 41.24, 40.97, 40.41, 40.69, 40.13, 39.58, 26.92, 23.37, 20.18; ESI-HRMS calculated for $C_{22}H_{32}Cl_2N_2$: 394.1943, Found: 322.2410 [M − 2HCl]$^+$; Anal. calcd. for $C_{22}H_{32}Cl_2N_2$: C, 66.83; H, 8.16; N, 7.08; Found: C, 66.64; H, 8.28; N, 7.12.

*6.1.3. N,N′-Bis-(3-methyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**15**)*

Yield: 85%; Mp: 229 °C; IR (Nujol, cm^{-1}): 2923, 2854, 2685, 2629, 1608, 1538, 1377, 1162, 803, 695; ^{1}H NMR (300 MHz, DMSO-d_6): 10.20 (brs, 2H), 9.87 (brs, 2H), 7.48–7.44 (m, 4H), 7.31–7.18 (m, 4H), 4.21 (d, J = 12 Hz, 2H), 4.07 (d, J = 12 Hz, 2H), 3.56 (s, 2H), 2.30 (s, 8H), 1.85–1.73 (m, 4H), 1.18 (s, 2 H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 138.56, 132.28, 131.77, 130.33, 129.30, 128.28, 56.86, 48.34, 26.48, 23.09, 21.78; ESI-HRMS calculated for $C_{22}H_{32}Cl_2N_2$: 394.1943, Found: 322.2408 [M − 2HCl]$^+$; Anal. calcd. for $C_{22}H_{32}Cl_2N_2$: C, 66.83; H, 8.16; N, 7.08; Found: C, 66.85; H, 8.30; N, 7.15.

*6.1.4. N,N′-Bis-(4-methyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**16**)*

Yield: 89%; Mp: 247 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2709, 2637, 1542, 1376, 1248, 1002, 817, 745; ^{1}H NMR (300 MHz, DMSO-d_6): 10.02 (s, 2H), 9.85 (s, 2H), 7.53–7.50 (d, J = 9 Hz, 4H), 7.21–7.18 (d, J = 9 Hz, 4H), 4.20 (d, J = 12 Hz, 2H), 4.06 (d, J = 12 Hz, 2H), 3.47 (s, 2H), 2.28 (s, 8H), 1.81–1.70 (m, 4H), 1.16–1.12 (s, 2H), ^{13}C NMR (75.5 MHz, DMSO-d_6): 139.23, 131.24, 129.95, 129.25, 56.75, 48.10, 26.59, 23.13, 21.64; ESI-HRMS calculated for $C_{22}H_{32}Cl_2N_2$: 394.1943, Found: 322.2412 [M − 2HCl]$^+$; Anal. calcd. for $C_{22}H_{32}Cl_2N_2$: C, 66.83; H, 8.16; N, 7.08; Found: C, 66.74; H, 8.34; N, 7.28.

*6.1.5. N,N′-Bis-(4-ethyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**17**)*

Yield: 89%; Mp: 218 °C; IR (KBr, cm^{-1}): 2932, 2717, 1545, 1461, 826; ^{1}H NMR (300 MHz, DMSO-d_6): 10.20 (brs, 2H), 9.83 (brs, 2H), 7.58 (d, J = 7.5 Hz, 4H), 7.25 (d, J = 7.5 Hz, 4H), 4.22 (d, J = 12.3 Hz, 2H), 4.09 (d, J = 12.3 Hz, 2H), 3.61 (s, 2H), 2.88 (q, J = 6.6 Hz, 4H), 2.34–2.31 (m, 2H), 1.88 (s, 4H), 1.16–1.18 (m, 8H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 149.19, 130.77, 130.57, 128.89, 126.39, 55.95, 47.31, 33.20, 25.60, 23.74, 23.67, 22.20; ESI-HRMS calculated for $C_{24}H_{36}Cl_2N_2$: 422.2256, Found: 350.2773 [M − 2HCl]$^+$; Anal. calcd. for $C_{24}H_{36}Cl_2N_2$: C, 68.07; H, 8.57, N, 6.62; Found: C, 68.20; H, 8.65, N, 6.54.

*6.1.6. N,N′-Bis-(4-isopropyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**18**)*

Yield: 86%; Mp: 232 °C; IR (KBr, cm^{-1}): 2959, 2870, 2714, 2631, 1579, 1517, 1461, 1363, 1267, 822; ^{1}H NMR (300 MHz, DMSO-d_6): 10.05 (brs, 2H), 9.80 (brs, 2H), 7.55 (d, J = 9 Hz, 4H), 7.23 (d, J = 9 Hz, 4H), 4.22 (d, J = 15 Hz, 2H), 4.08 (d, J = 15 Hz, 2H), 3.53 (s, 2H), 2.64–2.56 (m, 2H), 2.32–2.29 (m, 2H), 1.78 (s, 4H), 1.18–1.11 (m, 14H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 144.48, 144.68, 130.66, 130.49, 128.72, 127.93, 55.95, 48.49, 47.28, 27.90, 25.66, 22.23, 15.54; ESI-HRMS calculated for $C_{26}H_{40}Cl_2N_2$: 450.2569. Found: 378.3037 [M − 2HCl]$^+$; Anal. calcd. for $C_{26}H_{40}Cl_2N_2$: C, 69.16; H, 8.93; N, 6.20; Found: C, 69.28; H, 8.88; N, 6.34.

*6.1.7. N,N′-Bis-(4-n-butyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**19**)*

Yield: 82%; Mp: 200 °C; IR (KBr, cm^{-1}): 2931, 2858, 2633, 1545, 1519, 1458, 1006, 828, 524; ^{1}H NMR (300 MHz, DMSO-d_6): 10.16 (brs, 2H), 9.84 (brs, 2H), 7.56 (d, J = 9 Hz, 4H), 7.19 (d, J = 9 Hz, 4H), 4.20 (d, J = 12 Hz, 2H), 4.08 (d, J = 12 Hz, 2H), 3.57 (s, 2H), 2.56 (t, J = 9 Hz, 4H), 2.31–2.28 (m, 2H), 1.90–1.73 (m, 4H), 1.33–1.20 (m, 6H), 1.56–1.44 (m, 4H), 0.86 (t, J = 9 Hz, 6H); ^{13}C NMR (75.5 M Hz, DMSO-d_6): 13.70, 20.62, 21.63, 21.67, 22.21, 22.77, 23.91, 25.66, 25.87, 29.17, 32.98, 34.99, 47.27, 48.37, 50.21, 55.46, 55.89, 57.36, 128.37, 128.66, 128.93, 130.44, 143.17, 143.35; ESI-HRMS m/z [M]$^+$ calculated for $C_{28}H_{44}Cl_2N_2$: 478.2882. Found: 406.3349 [M − 2HCl]$^+$; Anal. calcd. for $C_{28}H_{44}Cl_2N_2$: C, 70.13; H, 9.25; N, 5.84; Found: C, 70.20; H, 9.22; N, 5.67.

*6.1.8. N,N′-Bis-(4-tert-butyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**20**)*

Yield: 90%; Mp: 244 °C; IR (Film, cm^{-1}): 2930, 2853, 1682, 1641, 1588, 1513, 1460, 1420, 1267, 1026; ^{1}H NMR (300 MHz, DMSO-d_6): 10.06 (brs, 2H), 9.73 (brs, 2H), 7.57 (d, J = 6 Hz, 4H), 7.41 (d, J = 9 Hz, 4H), 4.20 (d, J = 12 Hz, 2H), 4.08 (d, J = 12 Hz, 2H), 3.57 (s, 2H), 2.35–2.31 (m, 2H), 1.75 (s, 4H), 1.28–1.24 (s, 20H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 151.50, 130.26, 128.61, 125.31, 59.04, 47.19, 34.37, 31.01, 25.64, 22.23; ESI-HRMS m/z [M]$^+$ calculated for $C_{28}H_{44}Cl_2N_2$: 478.2882, Found: 406.3350 [M − 2HCl]$^+$; Anal. calcd. for $C_{28}H_{44}Cl_2N_2$: C, 70.13; H, 9.25; N, 5.84; Found: C, 70.24; H, 9.38; N, 5.88.

*6.1.9. N,N′-Bis-(2-bromo-benzyl)-cyclohexane-1,2-diaminium dichloride (**21**)*

Yield: 79%; Mp: 262 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2645, 1521, 1376, 1028, 805, 755, 670; ^{1}H NMR (300 MHz, DMSO-d_6): 10.25 (brs, 2H), 10.00 (brs, 2H), 7.97–7.93 (m, 2H), 7.69 (d, J = 7.8 Hz, 2H), 7.38–7.33 (m, 2H), 7.48–7.43 (m, 2H), 4.47–4.27 (m, 4H), 3.73 (s, 2H), 2.41–2.38 (m, 2H), 2.05–1.80 (m, 4H), 1.29 (s, 2H); ESI-HRMS m/z [M]$^+$ calculated for $C_{20}H_{26}Br_2Cl_2N_2$: 521.9840, Found: 450.0308 [M − 2HCl]$^+$, 452.0390 [M + 2 − 2HCl]$^+$, 454.0267 [M + 4 − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{26}Br_2Cl_2N_2$: C, 45.74; H, 4.99; N, 5.33; Found: C, 45.68; H, 4.85; N, 5.50.

*6.1.10. N,N′-Bis-(3-bromo-benzyl)-cyclohexane-1,2-diaminium dichloride (**22**)*

Yield: 85%; Mp: 242 °C; IR (KBr, cm^{-1}): 2939, 2862, 2689, 2622, 1728, 1570, 1472, 1372, 1246, 1209; ^{1}H NMR (300 MHz, DMSO-d_6): 10.33 (brs, 2H), 9.92 (brs, 2H), 7.99–7.96 (m, 2H), 7.74–7.68 (m, 2H), 7.59–7.56 (m, 2H), 7.38–7.33 (m, 2H), 4.33 (d, J = 12 Hz, 2H), 4.14 (d, J = 12 Hz, 2H), 3.63 (s, 2H), 2.28–2.32 (m, 2H), 1.97–1.76 (m, 4H), 1.20–1.13 (m, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 135.19, 133.95, 132.54, 131.39, 130.41, 122.40, 60.59, 57.17, 47.75, 26.42, 23.02, 21.62, 14.94; ESI-HRMS m/z [M]$^+$ calculated for $C_{20}H_{26}Br_2Cl_2N_2$: 521.9840, Found: 450.0310 [M − 2HCl]$^+$, 452.0388 [M + 2 − 2HCl]$^+$, 454.0268 [M + 4 − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{26}Br_2Cl_2N_2$: C, 45.74; H, 4.99; N, 5.33; Found: C, 45.60; H, 4.78; N, 5.45.

*6.1.11. N,N′-Bis-(4-bromo-benzyl)-cyclohexane-1,2-diaminium dichloride (**23**)*

Yield: 92%; Mp: 242 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2630, 1594, 1543, 1488, 1377, 1072, 1013, 808; ^{1}H NMR (300 MHz,

DMSO-d_6): 10.28 (s, 2H), 9.96 (s, 2H), 7.68–7.58 (m, 8H), 4.29 (d, J = 15 Hz, 2H), 4.12 (d, J = 15 Hz, 2H), 3.57 (s, 2H), 2.30–2.26 (m, 2H), 1.85–1.73 (m, 4H), 1.18–1.13 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 133.73, 133.55, 132.24, 131.80, 123.21, 60.59, 56.99, 49.04, 47.70, 26.49, 23.06, 21.62, 14.94; ESI-HRMS m/z $[M]^+$ calculated for $C_{20}H_{26}Br_2Cl_2N_2$: 521.9840, Found: 450.0308 $[M - 2HCl]^+$, 452.0390 $[M+2 - 2HCl]^+$, 454.0270 $[M + 4 - 2HCl]^+$; Anal. calcd. for $C_{20}H_{26}Br_2Cl_2N_2$: C, 45.74; H, 4.99; N, 5.33; Found: C, 45.50; H, 4.74; N, 5.48.

6.1.12. N,N′-Bis-(3-chloro-benzyl)-cyclohexane-1,2-diaminium dichloride (**25**)

Yield: 91%; Mp: 234 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2713, 2632, 1600, 1572, 1376, 1212, 1086, 883; ^{1}H NMR (300 MHz, DMSO-d_6): 10.33–10.06 (brs, 4H), 7.93 (m, 2H), 7.54–7.52 (m, 2H), 7.46–7.38 (m, 4H), 4.43 (d, J = 15 Hz, 2H), 4.28 (d, J = 15 Hz, 2H), 3.74 (s, 2H), 2.40–2.37 (m, 2H), 2.03–1.79 (m, 4H), 1.28–1.16 (m, 2H); ESI-HRMS m/z $[M]^+$ calculated for $C_{20}H_{26}Cl_4N_2$: 434.0850. Found: 362.3243 $[M - 2HCl]^+$, 364.1290 $[M + 2 - 2HCl]^+$, 363.1353 $[M + 4 - 2HCl]^+$; Anal. calcd. for $C_{20}H_{26}Cl_4N_2$: C, 55.06; H, 6.01; N, 6.42; Found: C, 55.49; H, 6.35; N, 6.48.

6.1.13. N,N′-Bis-(4-chloro-benzyl)-cyclohexane-1,2-diaminium dichloride (**26**)

Yield: 95%; Mp: 250 °C; IR (KBr, cm^{-1}): 2938, 2706, 1600, 1494, 1457, 1139, 1095, 1017, 814, 620; ^{1}H NMR (300 MHz, DMSO-d_6): 10.12 (brs, 4H), 7.66 (d, J = 6 Hz, 4H), 7.45 (d, J = 6 Hz, 4H), 4.28 (d, J = 12 Hz, 2H), 4.12 (d, J = 12 Hz, 2H), 3.74 (s, 2H), 2.25–2.29 (m, 2H), 1.72 (m, 4H), 1.19 (s, 2H); ESI-HRMS calculated for $C_{20}H_{26}Cl_4N_2$: 434.0850. Found: 362.3244 $[M - 2HCl]^+$, 364.1288 $[M + 2 - 2HCl]^+$, 363.1355 $[M + 4 - 2HCl]^+$; Anal. calcd. for $C_{20}H_{26}Cl_4N_2$: C, 55.06; H, 6.01; N, 6.42; Found: C, 55.23; H, 6.28; N, 6.69.

6.1.14. N,N′-Bis-(3-fluoro-benzyl)-cyclohexane-1,2-diaminium dichloride (**27**)

Yield: 92%; Mp: 237–238 °C; IR (KBr, cm^{-1}): 3042, 2936, 2826, 2713, 2635, 1618, 1590, 1545, 1459, 1253; ^{1}H NMR (300 MHz, DMSO-d_6): 10.37 (brs, 2H), 9.97 (brs, 2H), 7.65–7.62 (m, 2H), 7.50–7.46 (m, 4H), 7.23 (m, 2H), 4.35–4.22 (m, 4H), 3.62 (s, 2H), 2.28 (m, 2H), 1.87–1.76 (m, 4H), 1.37–1.20 (m, 2H); ESI-HRMS m/z $[M]^+$ calculated for $C_{20}H_{26}Cl_2F_2N_2$: 402.1441, Found: 330.1910 $[M - 2HCl]^+$; Anal. calcd. for $C_{20}H_{26}Cl_2F_2N_2$: C, 59.56; H, 6.50; N, 6.95; Found: C, 59.71; H, 6.58; N, 6.70.

6.1.15. N,N′-Bis-(2-nitro-benzyl)-cyclohexane-1,2-diaminium dichloride (**28**)

Yield: 89%; Mp: 260 °C; IR (Nujol, cm^{-1}): 2923, 2854, 2689, 1610, 1558, 1526, 1462, 1376, 1343, 1200; ^{1}H NMR (300 MHz, DMSO-d_6): 10.12 (brs, 4H), 8.16–8.14 (m, 2H), 8.01–7.99 (m, 2H), 7.82–7.78 (m, 2H), 7.71–7.66 (m, 2H), 4.57–4.46 (m, 4H), 3.76 (s, 2H), 2.37–2.33 (m, 2H), 2.02–1.81 (m, 4H), 1.47–1.33 (m, 2H); ESI-HRMS calculated for $C_{20}H_{26}Cl_2N_4O_4$: 456.1331, Found: 384.1795 $[M - 2HCl]^+$; Anal. calcd. for $C_{20}H_{26}Cl_2N_4O_4$: C, 52.52; H, 5.73; N, 12.25; Found: C, 52.28; H, 5.57; N, 12.48.

6.1.16. N,N′-Bis-(3-nitro-benzyl)-cyclohexane-1,2-diaminium dichloride (**29**)

Yield: 93%; Mp: 255 °C; R (KBr, cm^{-1}): 2930, 2733, 2646, 1608, 1537, 1460, 1350, 1106, 1024, 983; ^{1}H NMR (300 MHz, DMSO-d_6): 10.08 (brs, 4H), 8.58 (s, 2H), 8.24–8.15 (m, 4H), 7.72–7.67 (m, 2H), 4.49 (d, J = 12 Hz, 2H), 4.32 (d, J = 12 Hz, 2H), 3.61 (s, 2H), 2.34–2.37 (m, 2H), 1.79 (m, 4H), 1.28 (s, 2H); ESI-HRMS calculated for $C_{20}H_{26}Cl_2N_4O_4$: 456.1331, Found: 384.1793 $[M - 2HCl]^+$; Anal. calcd. for $C_{20}H_{26}Cl_2N_4O_4$: C, 52.52; H, 5.73; N, 12.25; Found: C, 52.35; H, 5.49; N, 12.35.

6.1.17. N,N′-Bis-(4-nitro-benzyl)-cyclohexane-1,2-diaminium dichloride (**30**)

Yield: 91%; Mp: 255–260 °C; IR (KBr, cm^{-1}): 2933, 1607, 1523, 1459, 1348, 857, 696; ^{1}H NMR (300 MHz, DMSO-d_6): 10.50 (brs, 2H), 10.12 (brs, 2H), 8.23 (d, J = 8.4 Hz, 4H), 7.96 (d, J = 8.4 Hz, 4H), 4.51 (d, J = 12 Hz, 2H), 4.32 (d, J = 12 Hz, 2H), 3.66 (s, 2H), 2.35–2.31 (m, 2H), 1.97–1.78 (m, 4H), 1.22–1.15 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 148.51, 140.12, 132.54, 124.19, 57.39, 48.99, 47.59, 26.48, 23.07; ESI-HRMS calculated for $C_{20}H_{26}Cl_2N_4O_4$: 456.1331, Found: 384.1291 $[M - 2HCl]^+$; Anal. calcd. for $C_{20}H_{26}Cl_2N_4O_4$: C, 52.52; H, 5.73; N, 12.25; Found: C, 52.76; H, 5.57; N, 12.41.

6.1.18. N,N′-Bis-(2-trifluoromethyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**31**)

Yield: 90%; Mp: 262 °C; IR (KBr, cm^{-1}): 2951, 2869, 2717, 2638, 1639, 1547, 1459, 1316, 1156, 1117; ^{1}H NMR (300 MHz, DMSO-d_6): 10.47–10.02 (m, 4H), 8.21 (d, J = 6 Hz, 2H), 7.80–7.73 (m, 4H), 7.64–7.59 (m, 2H), 4.50 (d, J = 15 Hz, 2H), 4.30 (d, J = 15 Hz, 2H), 3.82 (s, 2H), 2.37–2.33 (s, 2H), 20.3–1.80 (m, 4H), 1.30–1.16 (s, 2H); ESI-HRMS calculated for $C_{22}H_{26}Cl_2F_6N_2$: 502.1377, Found: 430.1845 $[M - 2HCl]^+$; Anal. calcd. for $C_{22}H_{26}Cl_2F_6N_2$: C, 52.50; H, 5.21; N, 5.57; Found: C, 52.63; H, 5.40; N, 5.74.

6.1.19. N,N′-Bis-(3-trifluoromethyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**32**)

Yield: 88%; Mp: 253 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2625, 1549, 1376, 1325, 1139, 1072, 815, 702; ^{1}H NMR (300 MHz, DMSO-d_6): 10.10 (brs, 4H), 8.10–8.01 (m, 4H), 7.74–7.64 (m, 4H), 4.43 (d, J = 12 Hz, 2H), 4.27 (d, J = 12 Hz, 2H), 3.63 (s, 2H), 2.35 (s, 2H), 1.80 (s, 4H), 1.27 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 135.56, 133.95, 130.29, 130.15, 129.73, 128.12, 126.70, 126.41, 123.09, 57.36, 47.94, 26.49, 23.03; ESI-HRMS calculated for $C_{22}H_{26}Cl_2F_6N_2$: 502.1377, Found: 430.1848 $[M - 2HCl]^+$; Anal. calcd. for $C_{22}H_{26}Cl_2F_6N_2$: C, 52.50; H, 5.21; N, 5.57; Found: C, 52.68; H, 5.35; N, 5.75.

6.1.20. N,N′-Bis-(4-trifluoromethyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**33**)

Yield: 86%; Mp: 255 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2616, 1551, 1376, 1326, 1164, 1127, 1068, 1021; ^{1}H NMR (300 MHz, DMSO-d_6): 10.10 (brs, 4H), 7.90 (d, J = 7.5 Hz, 4H), 7.75 (d, J = 7.5 Hz, 4H), 4.41 (d, J = 13.2 Hz, 2H), 4.26 (d, J = 13.2 Hz, 2H), 3.58 (brs, 2H), 2.31–2.35 (m, 2H), 1.77 (brs, 4H), 1.26 (brs, 2H); ESI-HRMS calculated for $C_{22}H_{26}Cl_2F_6N_2$: 502.1377, Found: 430.1843 $[M - 2HCl]^+$; Anal. calcd. for $C_{22}H_{26}Cl_2F_6N_2$: C, 52.50; H, 5.21; N, 5.57; Found: C, 52.48; H, 5.38; N, 5.40.

6.1.21. N,N′-Bis-(2,4-bis-trifluoromethyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**34**)

Yield: 87%; Mp: 260 °C; IR (KBr, cm^{-1}): 2933–2654, 1631, 1347, 1276, 1198, 1127, 850, 674; ^{1}H NMR (300 MHz, DMSO-d_6): 10.62–10.35 (brs, 4H), 8.45 (d, J = 6 Hz, 2H), 8.16–8.04 (m, 4H), 4.57 (d, J = 12 Hz, 2H), 4.33 (d, J = 12 Hz, 2H), 3.56 (s, 2H), 2.37–2.34 (m, 2H), 1.98–1.80 (m, 4H), 1.18–1.16 (s, 2H); ESI-HRMS calculated for $C_{24}H_{24}Cl_2F_{12}N_2$: 638.1125, Found: 566.1593 $[M - 2HCl]^+$; Anal. calcd. for $C_{24}H_{24}Cl_2F_{12}N_2$: C, 45.09; H, 3.78; N, 4.38; Found: 45.34; H, 3.45; N, 4.50.

6.1.22. N,N′-Bis-(2,5-bis-trifluoromethyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**35**)

Yield: 83%; Mp: 250 °C; IR (KBr, cm^{-1}): 2938, 2673, 1561, 1461, 1429, 1314,1183, 1124, 1093, 1043; ^{1}H NMR (300 MHz, DMSO-d_6): 10.64–10.46 (brs, 4H), 8.67 (s, 2H), 8.00–7.96 (m, 4H), 4.58 (d, J = 12 Hz, 2H), 4.39 (d, J = 12 Hz, 2H), 3.63 (s, 2H), 2.39–2.36 (m, 2H), 1.98–1.79 (m, 4H), 1.19–1.14 (m, 2H); ESI-HRMS calculated for

$C_{24}H_{24}Cl_2F_{12}N_2$: 638.1125, Found: 566.1592 [M − 2HCl]$^+$; Anal. calcd. for $C_{24}H_{24}Cl_2F_{12}N_2$: C, 45.09; H, 3.78; N, 4.38; Found: 45.32; H, 3.53; N, 4.25.

*6.1.23. N,N′-Bis-(4-methoxy-benzyl)-cyclohexane-1,2-diaminium dichloride (**36**)*

Yield: 90%; Mp: 235 °C; IR (Nujol, cm^{-1}): 2924, 2854, 2628, 1613, 1551, 1518, 1376, 1305, 1249, 1024; ^{1}H NMR (400 MHz, DMSO-d_6): 10.09 (brs, 2H), 9.86 (brs, 2H), 7.57 (d, J = 9 Hz, 4H), 6.93 (d, J = 6 Hz, 4H), 4.19–4.05 (m, 4H), 3.76 (s, 6H), 3.49 (m, 2H), 2.28–2.24 (m, 2H), 1.82–1.74 (m, 4H), 1.66 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 160.56, 132.89, 124.03, 114.76, 56.55, 56.06, 49.07, 47.87, 26.56, 23.08; ESI-HRMS calculated for $C_{22}H_{32}Cl_2N_2O_2$: 426.1841, Found: 354.4860 [M − 2HCl]$^+$; Anal. calcd. for $C_{22}H_{32}Cl_2N_2O_2$: C, 61.82; H, 7.55; N, 6.55; Found: C, 61.60; H, 7.40; N, 6.75.

*6.1.24. (1S,2S)-N,N′-Bis-(3-bromo-benzyl)-cyclohexane-1,2-diaminium dichloride (**37**)*

Yield: 88%; Mp: 265 °C; IR (KBr, cm^{-1}): 2933, 2654, 1631, 1461, 1347, 1276, 1198, 1127, 1089, 915; ^{1}H NMR (400 MHz, DMSO-d_6): 10.18 (brs, 2H), 9.81 (brs, 2H), 7.95 (s, 2H), 7.70–7.58 (m, 4H), 7.40–7.35 (m, 2H), 4.34 (d, J = 12 Hz, 2H), 4.15 (d, J = 12 Hz, 2H), 3.60(s, 2H), 2.29 (s, 2H), 1.75 (s, 4H), 1.22 (s, 2H); ESI-HRMS m/z [M]$^+$ calculated for $C_{20}H_{26}Br_2Cl_2N_2$: 521.9840. Found: 450.0309 [M − 2HCl]$^+$, 452.0391 [M + 2 − 2HCl]$^+$, 454.0272 [M + 4 − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{26}Br_2Cl_2N_2$: C, 45.74; H, 4.99; N, 5.33; Found: C, 45.49; H, 4.75; N, 5.58.

*6.1.25. (1S,2S)-N,N′-Bis-(4-bromo-benzyl)-cyclohexane-1,2-diaminium dichloride (**38**)*

Yield: 93%; Mp: 260-265 °C; IR (KBr, cm^{-1}): 2943, 2703, 2622, 1594, 1544, 1490, 1456, 1072, 1013, 807; ^{1}H NMR (300 MHz, DMSO-d_6): 10.11 (brs, 2H), 9.86 (brs, 2H), 7.62 (s, 8H), 4.30 (d, J = 12 Hz, 2H), 4.13 (d, J = 12 Hz, 2H), 3.53 (s, 2H), 2.31–2.28 (m, 2H), 1.73 (s, 4H), 1.20 (s, 2H); ESI-HRMS calculated for $C_{20}H_{26}Br_2Cl_2N_2$: 521.9840. Found: 450.0310 [M − 2HCl]$^+$, 452.0397 [M + 2 − 2HCl]$^+$, 454.0275 [M + 4 − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{26}Br_2Cl_2N_2$: C, 45.74; H, 4.99; N, 5.33; Found: C, 45.50; H, 4.73; N, 5.57.

*6.1.26. (1S,2S)-N,N′-Bis-(3-trifluoromethyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**39**)*

Yield: 90%; IR (KBr, cm^{-1}): 2942, 2870, 2713, 2631, 1561, 1455, 1331, 1207, 1166, 1121; ^{1}H NMR (400 MHz, DMSO-d_6): 10.18 (brs, 2H), 9.92 (brs, 2H), 8.12 (s, 2H), 8.01–7.99 (m, 2H), 7.77–7.75 (m, 2H), 7.68–7.63 (m, 2H), 4.46 (d, J = 12 Hz, 2H), 4.27 (d, J = 12 Hz, 2H), 3.64 (s, 2H), 2.37–2.34 (m, 2H), 1.79 (s, 4H), 1.25 (s, 2H); ESI-HRMS calculated for $C_{22}H_{26}Cl_2F_6N_2$: 502.1377. Found: 430.1844 [M − 2HCl]$^+$; Anal. calcd. for $C_{22}H_{26}Cl_2F_6N_2$: C, 52.50; H, 5.21; N, 5.57; Found: C, 52.67; H, 5.44; N, 5.32.

*6.1.27. (1S,2S)-N,N′-Bis-(4-trifluoromethyl-benzyl)-cyclohexane-1,2-diaminium dichloride (**40**)*

Yield: 94%; Mp: 268 °C; IR (KBr, cm^{-1}): 2932, 1621, 1544, 1458, 1327, 1165, 1128, 1068, 828; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.47 (brs, 2 H), 10.07 (brs, 2H), 7.92 (d, J = 6 Hz, 4H), 7.75 (d, J = 6 Hz, 4H), 4.46 (d, J = 12 Hz, 2H), 4.28 (d, J = 12 Hz, 2H), 3.68 (s, 2H), 2.36–2.33 (m, 2H), 1.78 (s, 4H), 1.22 (s, 2H); ESI-HRMS calculated of $C_{22}H_{26}Cl_2F_6N_2$: 502.1377. Found: 430.1848 [M − 2HCl]$^+$; Anal. calcd. for $C_{22}H_{26}Cl_2F_6N_2$: C, 52.50; H, 5.21; N, 5.57; Found: C, 52.75; H, 5.42; N, 5.40.

*6.1.28. (1S,2S)-N,N′-Bis-(4-chloro-benzyl)-cyclohexane-1,2-diaminium dichloride (**41**)*

Yield: 89%; Mp: 255-260 °C; IR (KBr, cm^{-1}): 2941, 2870, 2707, 2631, 1493, 1458, 1093, 1016, 812, 517; ^{1}H NMR (400 MHz, DMSO-d_6): 10.28 (brs, 2H), 9.94 (brs, 2H), 7.70 (d, J = 9 Hz, 4H), 7.46 (d, J = 9 Hz, 4H), 4.32 (d, J = 12 Hz, 2H), 4.15 (d, J = 12 Hz, 2H), 3.57(s, 2H), 2.31–2.28 (m, 2H), 1.74 (s, 4H), 1.19 (s, 2H); ESI-HRMS calculated for $C_{20}H_{26}Cl_4N_2$: 434.0850. Found: 362.3242 [M − 2HCl]$^+$, 364.1285 [M + 2 − 2HCl]$^+$, 363.1353 [M + 4 − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{26}Cl_4N_2$: C, 55.06; H, 6.01; N, 6.42; Found: C, 55.30; H, 6.25; N, 6.59.

*6.1.29. (±)-trans-N,N′-Bis-(2,4-dichloro-benzyl)-cyclohexane-1,2-diaminium dichloride (**42**)*

Yield: 93%; Mp: 255–260 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 10.20 (brs, 2H), 9.83 (brs, 2H), 7.93 (s, 1H), 7.64 (d, J = 8 Hz, 2H), 7.54 (d, J = 8 Hz, 2H), 7.35–7.31 (m, 1H), 4.30 (d, J = 9 Hz, 2H), 4.11 (d, J = 9 Hz, 2H), 3.58 (s, 2H), 2.28–2.25 (m, 2H), 1.73 (m, 4 H), 1.17 (s, 2H); ^{13}C NMR (75.5 MHz,. DMSO-d_6): IR (Nujol): 2924, 2855, 2623, 1553, 1460, 1377, 1144 cm^{-1}; ESI-HRMS calculated for $C_{20}H_{24}Cl_6N_2$: 502.0071 Found: 430.0540 [M − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{24}Cl_6N_2$: C, 47.55; H, 4.79; N, 5.55; Found: C, 47.43; H, 4.57; N, 5.43.

*6.1.30. (±)-trans-N,N′-Bis-(3-bromo-benzyl)-cyclohexane-1,2-diaminium dichloride (**43**)*

Yield: 90%; Mp: 237–240 °C; IR (KBr, cm^{-1}): 2921, 2863, 2633, 1463, 1377, 1078, 875, 811; ^{1}H NMR (300 MHz, DMSO-d_6): 10.20 (brs, 2H), 9.83 (brs, 2H), 7.92 (s, 2H), 7.65 (d, J = 7 Hz, 2H), 7.54 (d, J = 8 Hz, 2H), 7.35–7.31 (m, 2H), 4.30 (d, J = 10 Hz, 2H), 4.11 (d, J = 10 Hz, 2H), 3.58 (s, 2H), 2.55–2.28 (m, 2H), 1.73 (m, 4H), 1.17 (m, 2H); ESI-HRMS calculated for $C_{20}H_{26}Br_2Cl_2N_2$: 521.9840, Found: 450.0312 [M − 2HCl]$^+$, 452.0390 [M + 2 − 2HCl]$^+$, 454.0265 [M + 4 − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{26}Br_2Cl_2N_2$: C, 45.74; H, 4.99; N, 5.33; Found: C, 45.40; H, 4.58; N, 5.55.

*6.1.31. (±)-trans-N,N′-Bis-(3-chloro-benzyl)-cyclohexane-1,2-diaminiumchloride (**44**)*

Yield: 85%; Mp: 240–245 °C; IR (Nujol, cm^{-1}): 2924, 2852, 2630, 1458, 1377, 1209, 868, 805; ^{1}H NMR (400 MHz, DMSO-d_6): 9.98 (brs, 2H), 9.73 (brs, 2H), 7.80 (m, 2H), 7.60–7.58 (m, 2H), 7.43–7.39 (m, 4H), 4.31 (d, J = 9 Hz, 2H), 4.28 (d, J = 8 Hz, 2H), 3.52 (s, 2H), 2.29–2.26 (m, 2H), 1.71 (m, 4H), 1.21–1.19 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 134.57, 133.55, 130.75, 129.68, 129.33, 56.86, 47.45, 26.07, 22.64; ESI-HRMS calculated for $C_{20}H_{26}Cl_4N_2$: 434.0850. Found: 362.3273 [M − 2HCl]$^+$, 364.1279 [M + 2 − 2HCl]$^+$, 363.1344 [M + 4 − 2HCl]$^+$; Anal. calcd. for $C_{20}H_{26}Cl_4N_2$: C, 55.06; H, 6.01; N, 6.42; Found: C, 55.29; H, 6.30; N, 6.34.

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