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Synthesis, characterization, and anti-bacterial efficacy of some novel cyclophane amide

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Abstract—Synthesis of macrocyclic di, tetra- and hexaamides with aza and oxy linkages has been achieved and the inhibitory activity of cyclophane amides against human pathogenic bacteria well documented.

1. Introduction

The increasing interest in the synthesis of aza crown compounds due to their important diverse application in supramolecular chemistry[1–5] stimulates the imaginative skill of synthetic chemists. Various changes have been made to the basic crown ether structure in order to enhance the selectivity of the ligands and the stability of complexes formed with both metal and organic cations and to use them as models of protein–metal binding sites in biological systems,[6–8] synthetic ionophores, therapeutic reagents in chelate therapy, cyclic antibiotics,[9] to study host–guest interactions[10] and in catalysis.[11] Some of these modifications involve the substitution of the ligand polyether oxygen donor atoms by sulfur and/or nitrogen atoms.[12] Other substitution involved the insertion of functional groups viz., amides, esters in the ring.[13–16] Recognition of the importance of macrocyclic compounds has led to considerable effort being invested in developing reliable inexpensive synthetic routes to these compounds.

The biphenyl based cyclic amides have been reported for anion complexation.[17] Cyclic tetraamide receptors having barbiturate binding domain were reported.[18] Supramolecular amides are also used as molecular receptors[19] and in molecular recognition[20] of biologically interacting substrates including anti-HIV active macrocyclic amides.[21] Synthesize of macrocyclic hexaamides and their bonding properties with peptides has been also reported.[22] The self-assembly of acyclic peptides and hence their ability to form β-sheet structures has been demonstrated.[23,24] Adamantane based systems also form double-helical cyclic structures.[25] Cyclic peptide[26] with open pores is useful as transport vehicles for biologically important ions[27] or neutral molecules.[28] The increased anti-bacterial and anti-fungal activity of copper complexes of macrocyclic compounds than the uncomplexed macro cyclic compounds has been reported.[29] Tetra aza macrocyclic complexes have been screened for their biological activity.[30] Hence it is of interest to synthesize and study the anti-bacterial activity of some novel cyclophane amides. We wish to report the synthesize of cyclophane amides **7**–**11**, **13**–**16**, **17**, **18**, and **21** and the anti-bacterial efficacy of some of the cyclophane amides against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Proteus vulgaris* bacteria.

2. Results and discussion

Diacid chlorides **1**–**5** were prepared by the reaction of the corresponding dicarboxylic acid and thionyl chloride and used for the synthesize of cyclophane amides.

Diamide **6** required for the synthesize of cyclophane amide was obtained in 60% yield by the reaction of diphenic acid chloride **4** with 2.1 equiv of *N*-phenyl ethylene diamine (NPEDA) in presence of triethyl amine in methylene chloride (Scheme 1).

Keywords: Cyclophane tetraamide; Ion transportation; Host–guest interaction.
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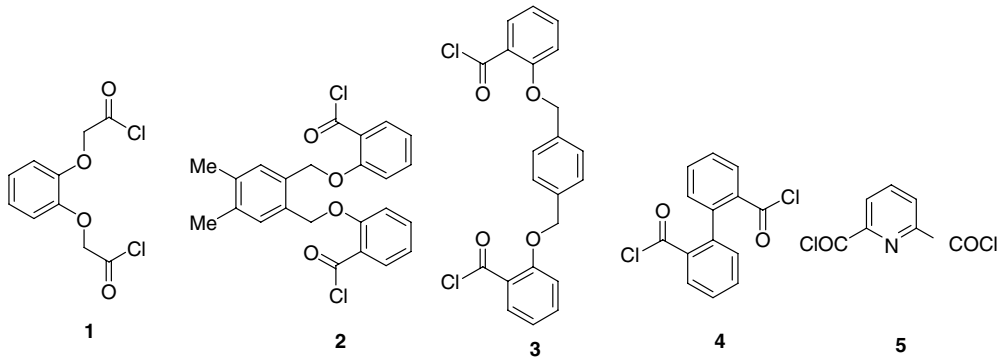
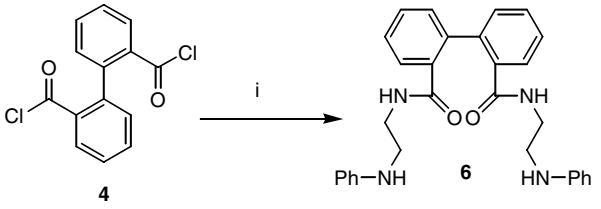


Diamide **6** in the ^{1}H NMR spectrum displayed –NCH_2CH_2N– protons as multiplet at δ 2.87–3.50, and a broad singlet at δ 4.30 for –NHPh proton in addition to the aromatic protons at δ 6.45–7.45 and the amide proton at δ 9.90 as a broad singlet. The structure of the diamide **6** was confirmed based on spectral and analytical data.

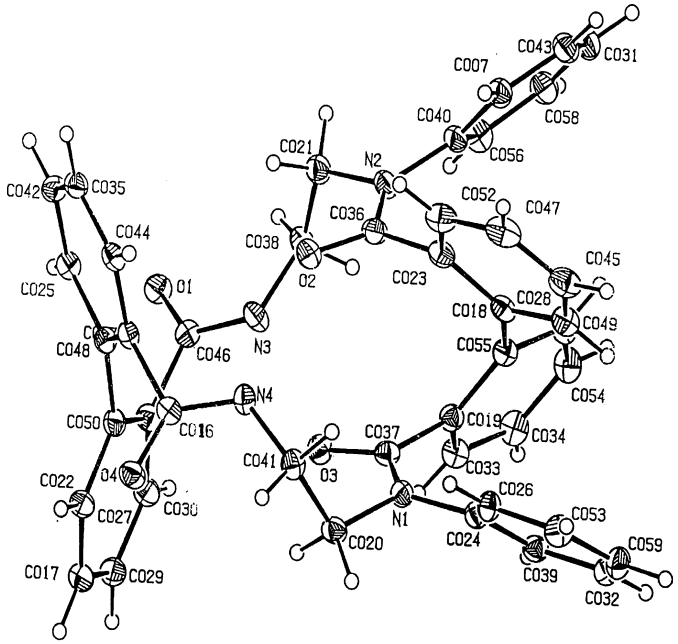
Reaction of the diamide **6** with diacid chlorides **1**–**5** in the presence of triethyl amine under high dilution condition at reflux in chloroform gave cyclophane tetraamides **7**–**11** in 33–40% yield (Scheme 2).

Cyclophane amide **8** in ^{1}H NMR spectrum displayed the aromatic methyl protons as a singlet at δ 2.36, –NCH_2CH_2N– protons as a multiplet at δ 3.18–3.72, –OCH_2 protons as an AB quartet at δ 5.00 and 5.17 with J = 13.2 Hz and the amide protons as a broad singlet at δ 8.26 in addition to the aromatic protons. In ^{13}C NMR spectrum, two aromatic methyl carbons appeared at δ 19.8, –NCH_2CH_2N–, –OCH_2 and carbonyl carbon appeared at δ 38.6, 47.9, 68.7, and 170.4, respectively, in addition to the aromatic carbons. Further the structures of the cyclophane amide **8** was confirmed by the appearance of the molecular ion at *m*/*z* 848. Similarly the structure of the cyclophane amides **7**, **9**, **10**, and **11** were thoroughly characterized by spectral and analytical data. Cyclophane amide **7** showed carbonyl stretching frequency at 1677 cm^{-1} in IR spectrum. The reason for the higher value compared to other cyclophane amides is possibly due to the attachment of the carbonyl group to the methylene group, where as in the other cyclophane amide it is attached to the aromatic ring. The structure of cyclophane tetraamide **10** was also confirmed by XRD.

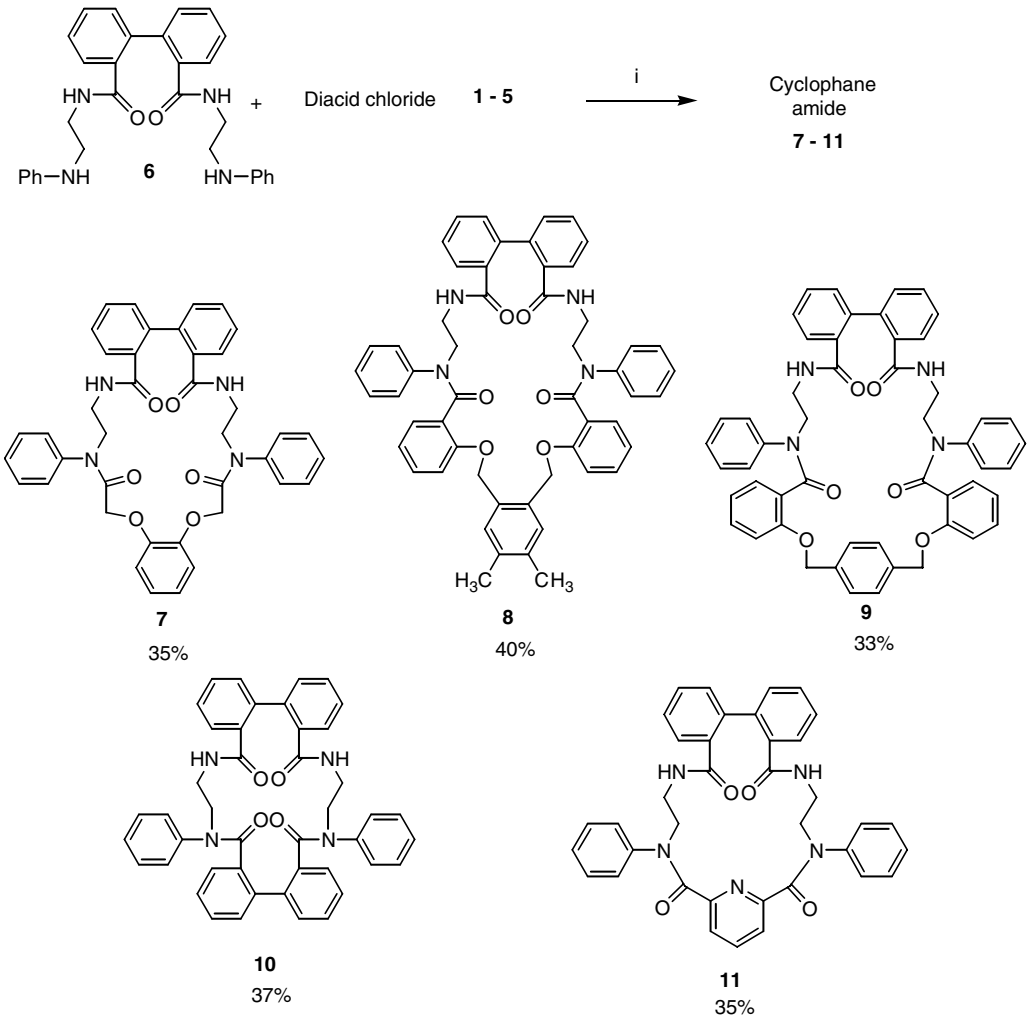


Scheme 1. Reagents and condition: (i) 2.1 equiv of NPEDA, TEA, CH_2Cl_2, rt, 1 h.

With a view to synthesize cyclophane amides of smaller cavity, dichloro compound **12** was prepared by the reaction of OPDA with two equivalents of chloro acetyl chloride in the presence of TEA in methylene chloride. Reaction of the dichloro compound **12** with various

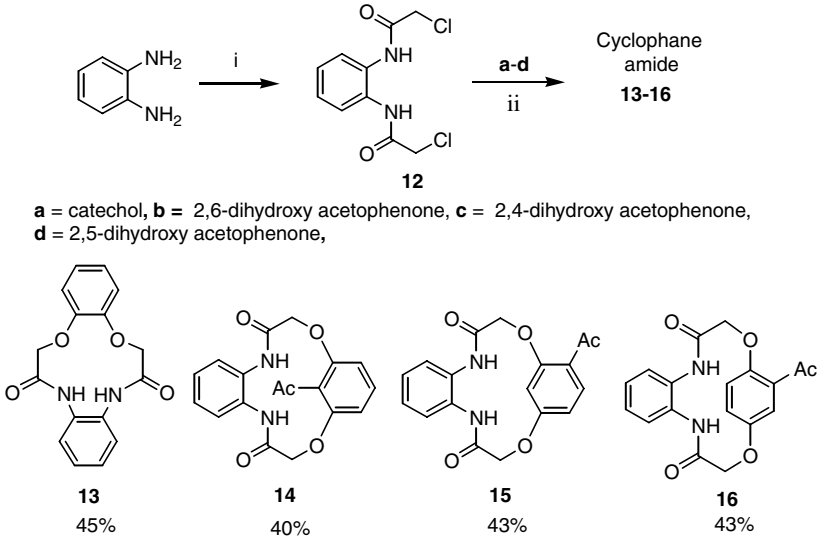


ORTEP diagram of cyclophane tetraamide **10**



Scheme 2. Reagents and condition: (i) TEA, chloroform, reflux, 6 h.

dihydroxy benzene derivatives viz., catechol, 2,6-dihydroxy acetophenone, 2,4-dihydroxy acetophenone, and 2,5-dihydroxy acetophenone in the presence of anhydrous potassium carbonate and KI in acetonitrile under reflux for 12 h gave cyclophane diamides **13**–**16** in 40–45% yield (Scheme 3).



Scheme 3. Reagents and condition: (i) 2.1 equiv of chloro acetylchloride, TEA, CH_2Cl_2; (ii) K_2CO_3, Kl, CH_3CN, reflux, 12 h.

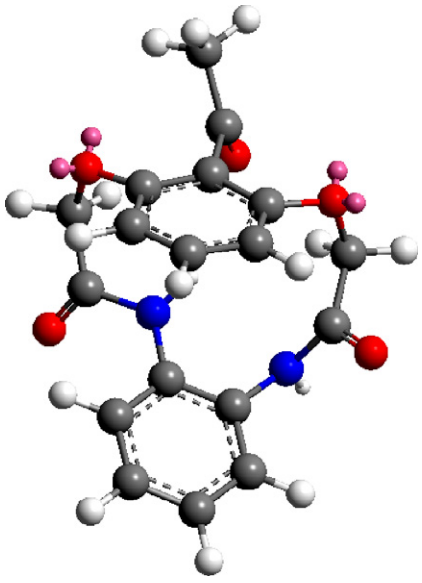


Figure 1. Energy minimization of cyclophane amide **14**. Heat of formation of cyclophane amide **14** is −0.8023 kcal/mol.

In the ^{1}H NMR spectrum diamide **15** displayed the $-COCH_3$ protons as a singlet at δ 2.54, $-OCH_2$ protons as two singlets at δ 4.33 and 5.11 and NH protons as two singlets at δ 10.72 and 12.56 in addition to the aromatic protons. In the ^{13}C NMR spectrum, methyl and methylene carbons appeared at δ 26.6 and 65.7 and the carbonyl carbons appeared at δ 163.8, 164.2, and 166.2 in addition to the aromatic carbons. In the mass spectrum the molecular ion appeared at *m/z* 340. Thus, the structure of cyclophane **15** was confirmed based on spectral and analytical data. Similarly structures of the cyclophane **13**, **14**, and **16** was also characterized by spectral and analytical data.

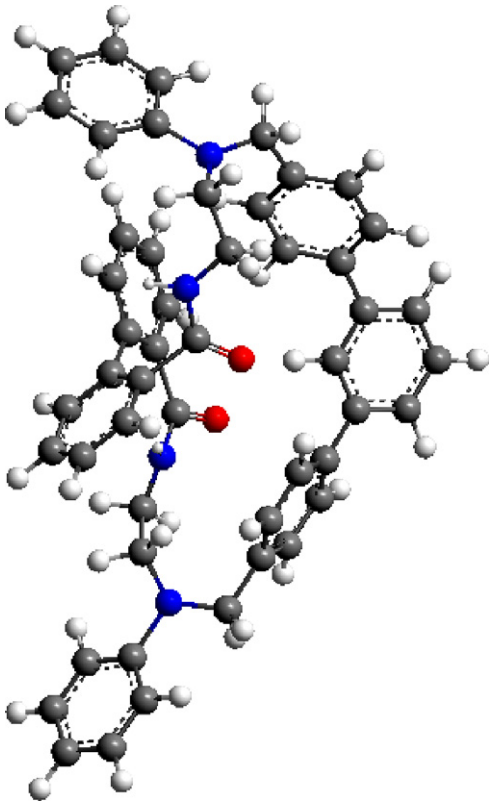
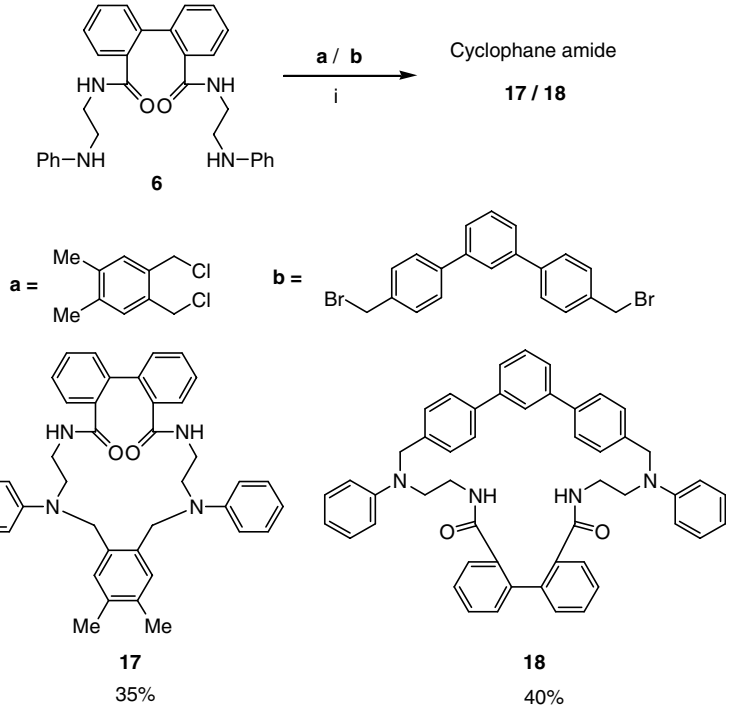
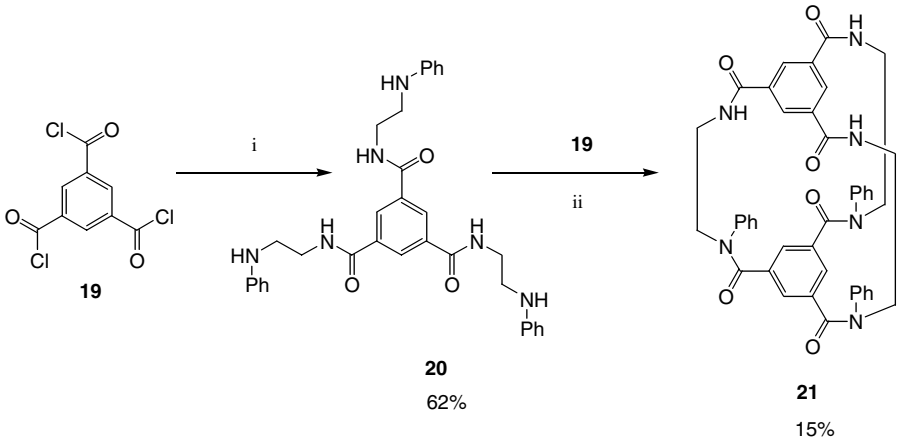


Figure 2. Energy minimization of cyclophane amide **18**. Heat of formation of cyclophane amide **18** is 5.5746 kcal/mol.

Structure of the cyclophane **14** would be interesting with the possibility of acetyl group oriented in the cavity due



Scheme 4. Reagents and condition: (i) K_2CO_3, CH_3CN, reflux, 24 h.



Scheme 5. Reagents and condition: (i) 3.1 equiv of NPEDA, TEA, CH_2Cl_2, rt, 1 h; (ii) TEA, $CHCl_3$, reflux, 6 h.

to the hydrogen bonding with the NH group. Hence energy minimization (MM2) calculations were carried out on the cyclophane amide **14**, which reveals that the acetyl group is projecting out of the cavity (Fig. 1). Such preferred orientation could be due to the smaller cavity, which pushes the acetyl group out of the cavity.

In order to synthesize cyclophane amides with aza linkages, the diamide **6** was reacted with 4,5-bis (chloromethyl) *o*-xylene and 1,3-bis (4-bromomethyl phenyl) benzene in presence of anhyd K_2CO_3 in acetonitrile under reflux for 24 h to give cyclophane diamides **17** and **18** in 35% and 40% yield, respectively (Scheme 4).

Cyclophane **17** in the ^{1}H NMR spectrum displayed aromatic methyl protons atδ 2.15, $-NCH_2CH_2N-$ protons as a multiplet at δ 3.11–3.89, $-NCH_2$ protons at δ 4.49 and amide protons as a broad singlet at δ 8.04 in addition to the aromatic protons. In the ^{13}C NMR spectrum, the aromatic methyl carbons, $-NCH_2CH_2N-$ carbons, $-NCH_2$ carbons and carbonyl carbon appeared at δ 19.5, 38.9, 49.8, 51.3, and 170.0 in addition to the aromatic carbons and in the mass spectrum the molecular ion appeared at *m*/*z* 608. Similarly cyclophane **18** was also characterized by spectral and analytical data.

The presence of *m*-terphenyl group should increase the cavity size if the cyclophane amide **18** is really rigid. Hence MO calculation (MM2) was carried out on cyclophane amide **18**. However, the molecule adopted concave nature and the biphenyl unit is twisted due to the strain caused by the presence of large *m*-terphenyl group (Fig. 2).

With a view to synthesize bicyclic hexaamide **21**, 1,3,5-benzene tricarboxylic acid chloride **19** was reacted with three equivalents of *N*-phenyl ethylene diamine in presence of TEA in methylene dichloride to give triamide **20** in 62% yield. In ^{1}H NMR spectrum, triamide **20** displayed $-NCH_2CH_2N-$ protons as multiplet at δ 3.22–3.46, aromatic protons of the tricarbonyl benzene ring as a singlet at δ 8.44 and the amide protons appeared as a broad singlet at δ 8.82 in addition to the aromatic protons. In mass spectrum the triamide **20** displayed the molecular ion at *m*/*z* 564.

Triamide **20** was reacted with 1,3,5-benzene tricarboxylic acid chloride **19** in presence of TEA under very high dilution conditions at reflux for 6 h to give bicyclic hexaamide **21** in 15% yield (Scheme 5). Hexaamide **21** in pure form was insoluble in usual NMR solvents; hence NMR spectrum could not be recorded. However, cyclophane **21** in FAB mass spectrum showed the molecular ion at *m*/*z* 720.

The 3D structure of the cyclophane amide **21** would be interesting and there is a possibility for a cylindrical cavity with the two trisubstituted aromatic rings as spacer units. However, when MO calculation (MM2) was car-

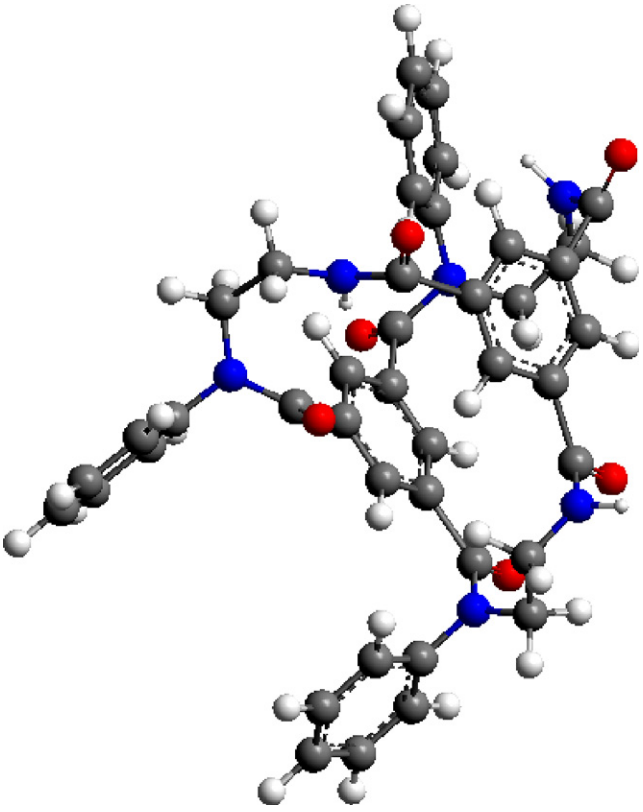


Figure 3. Energy minimization of bicyclic amide **21**. Heat of formation of bicyclic amide **21** is −3.1345 kcal/mol.

ried out on cyclophane **21**, it revealed that the two aromatic spaces are nearly perpendicular to each other. This shows that the molecule is not rigid and the dimension of the cavity is totally controlled by the groups present in the pillar units [$CONPhCH_2CH_2NHCO$] (Fig. 3).

Similarly attempts were made to synthesize triamide **24** from diamine **23** which was synthesized by the reaction of α-chloroacetanilide with 2-aminothiophenol in methanolic KOH at rt for 4 h and subsequent reduction of the resulting amide compound **22** with sodium borohydride (excess) in presence of acetic acid in THF at reflux for 6 h in 48% overall yield. Compound **22** in ^1H NMR spectrum displayed $–SCH_2$ and $–NH_2$ protons at δ 3.60 and 4.60 and the amide –NH protons appeared at δ 9.20 in addition to aromatic protons. Diamine **23** in ^1H NMR spectrum displayed $–SCH_2$ protons as a triplet at δ 3.00 with $J = 4.2$ Hz and $–NCH_2$ protons as multiplet at δ 3.30, in addition to aromatic protons at δ 6.30 to 7.40. The $–NH_2$ and –NH protons appeared as broad singlet at δ 4.10. Reaction of 1,3,5-benzene tricarboxylic acid chloride **19** with 3.1 equivalents of diamine **23** in presence of TEA in methylene dichloride gave complex reaction mixture and did not yield precyclophane **24** (Scheme 6).

2.1. Anti-bacterial activity

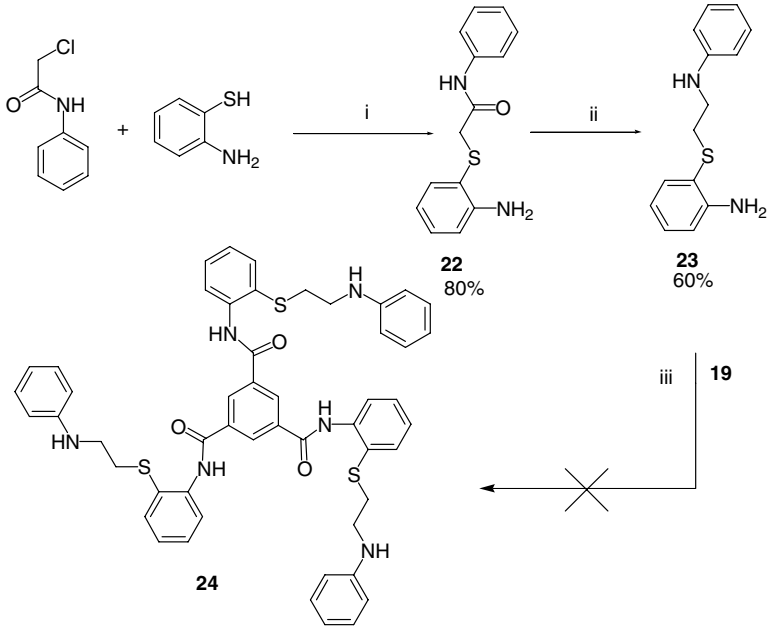
The bactericidal activity of the cyclophane amides **7**–**9**, **13**, **15**–**18**, and **21** was assayed against *E. coli*, *S. aureus*, *St. faecalis*, and *P. vulgaris* by disc diffusion method. All cyclophane amides inhibited the growth of *E. coli*. Cyclophane amides **7** and **8** exhibited significant activity towards *E. coli*. All cyclophane amides inhibited the growth of *S. aureus* except for cyclophane amide **17** at a concentration of 50 μg/mL of dimethylsulfoxide (DMSO). Since the minimum inhibitory concentration [MIC] for cyclophane amide **17** is more than 50 μg/mL against *S. aureus*, it did not inhibit the growth. Cyclophane amides **8** and **21** exhibited significant activity towards *S. aureus*. All cyclophane amides inhibited the growth of *St. faecalis* except for cyclophane amide **17** at a concentration of 50 μg/mL, since the minimum inhibitory concentration for cyclophane amide **17** is more than 50 μg/mL against *St. faecalis*. Cyclophane

Table 1. Inhibition effects of cyclophane amides on the growth of *Escherichia coli*

Cyclophane	Zone of inhibition (mm)		
	50 μg/mL	100 μg/mL	150 μg/mL
7	10	12	14
8	11	13	14
9	9	11	12
13	8	10	12
15	8	11	12
16	7	8	10
17	8	10	11
18	11	12	13
21	8	9	10

Table 2. Inhibition effects of cyclophane amides on the growth of *Staphylococcus aureus*

Cyclophane	Zone of inhibition (mm)		
	50 μg/mL	100 μg/mL	150 μg/mL
7	7	9	11
8	12	14	15
9	7	8	10
13	8	9	11
15	7	9	11
16	7	8	9
17	—	7	9
18	10	12	13
21	10	12	14



Scheme 6. Reagents and conditions: (i) KOH, MeOH, rt, 4 h; (ii) $NaBH_4$, AcOH, THF, reflux, 6 h; (iii) TEA, CH_2Cl_2, rt, 1 h.

Table 3. Inhibition effects of cyclophane amides on the growth of *Streptococcus faecalis*

Cyclophane	Zone of inhibition (mm)		
	50 μg/mL	100 μg/mL	150 μg/mL
7	7	9	10
8	8	10	12
9	6	7	9
13	7	8	9
15	8	10	11
16	8	9	12
17	—	9	10
18	12	13	14
21	6	7	8

Table 4. Inhibition effects of cyclophane amides on the growth of *Proteus vulgaris*

Cyclophane	Zone of inhibition (mm)		
	50 μg/mL	100 μg/mL	150 μg/mL
7	10	12	13
8	12	14	15
9	11	12	13
13	8	9	10
15	—	7	8
16	9	10	11
17	—	8	9
18	9	11	12
21	7	8	10

18 exhibited significant activity towards *St. faecalis.* All cyclophane amides inhibited the growth of *P. vulgaris* except for cyclophane amides **15** and **17** at a concentration of 50 μg/mL, since the MIC for cyclophane amides **15** and **17** is more than 50 μg/mL against *P. vulgaris*. Cyclophane **8** exhibited significant activity towards *P. vulgaris.* The standard antibiotic disc (*Streptomycin* 10 μg/ disc) inhibited the growth of *E. coli* by 12 mm, *S. aureus* by 15 mm, *St. faecalis* by 13 mm and *P. vulgaris* by 12 mm, respectively. The diameter of inhibition zone for each concentration against all the test bacteria is depicted in Tables 1–4.

3. Conclusion

In conclusion, cyclophane diamides, cyclophane tetraamides, and bicyclic hexaamide have been synthesized, characterized and the bactericidal activity of the cyclophane amides **7**–**9**, **13**, **15**–**18**, and **21** was assayed against *E. coli*, *S. aureus*, *St. faecalis*, and *P. vulgaris* by disc diffusion method. Cyclophane amides **7**, **8**, and **18** could be used as potential compounds to ward-off the diseases caused by these bacteria.

4. Experimental

4.1. General directions

All ^{1}H NMR and ^{13}C NMR were recorded in $CDCl_3$ and DMSO-d_6 with JEOL Model: GSX 400. EI-MS spectra were recorded using JEOL DX-303 mass spectrometer and FAB MS spectra were recorded using JEOL SX 102/DA-6000 mass spectrometer using a *m*-nitro benzyl alcohol (NBA) matrix. Melting points were recorded with Gallenkamp melting point apparatus. Pre-coated silica gel plates from Merck were used for TLC. Column chromatography was carried out using silica gel (60–120 mesh) purchased from Acme.

4.2. General procedure for the preparation of diamide 6 and triamide 20

Diphenic acid chloride **4** (1.5 mmol)/1,3,5-benzene tricarboxylic acid chloride **19** (1.0 mmol) in methylene chloride (10 mL) was added to a mixture of *N*-phenyl ethylene diamine (3.0 mmol), TEA (3.0 mmol) in methylene chloride (30 mL) for 1 h at room temperature after which the reaction mixture was washed with water and dried over anhyd $MgSO_4$. After removing the solvent under vacuum, the residue was chromatographed over SiO_2 to give diamide **6**/triamide **20**.

4.2.1. Diamide 6. Yield: 60% (430 mg); mp 180–183 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 2.87–3.50 (m, 8H), 4.30 (br s, 2H), 6.45–7.45 (m, 18H), 9.90 (br s, 2H); IR (KBr, cm^{-1}): 3342, 2944, 1664, 1530; MS (EI): *m*/*z*: 478; Elemental Anal. calcd for $C_{30}H_{30}N_4O_2$: C, 75.30; H, 6.27; N, 11.7. Found: C, 75.27; H, 6.11; N, 11.6.

4.2.2. Triamide 20. Yield: 62% (350 mg); mp 198–201 °C; eluent for column chromatography: $CHCl_3$/MeOH (19:1); ^{1}H NMR: (400 MHz, DMSO-d_6) δ 3.22–3.46 (m, 12H), 5.70 (br s, 3H), 6.50–7.08 (m, 15H), 8.44 (s, 3H), 8.82 (br s, 3H); IR (KBr, cm^{-1}): 3342, 2944, 1654,1521; MS (EI): *m*/*z*: 564; Elemental Anal. calcd for $C_{33}H_{36}N_6O_3$: C, 70.19; H, 6.43; N, 14.8. Found: C, 70.22; H, 6.32; N, 14.6.

4.3. General procedure for the synthesize of cyclophane tetraamides 7–11

A solution of the diacid chloride (0.5 mmol) in dry chloroform (100 mL) and a solution of the diamine (0.5 mmol) and triethyl amine (1.1 mmol) in dry chloroform (100 mL) were simultaneously added dropwise during 6 h to a well-stirred solution of chloroform (500 mL) at reflux. After the addition was complete, the reaction mixture was refluxed for another 6 h. The solvent was removed at reduced pressure and the residue obtained was then dissolved in chloroform (300 mL), washed with water (2× 100 mL) to remove the triethylammonium chloride and dried over magnesium sulfate. Removal of the chloroform gave the cyclophane as a crude material, which was purified by column chromatography (SiO_2) with suitable eluting solvent as mentioned under each cyclophane.

4.3.1. Cyclophane tetraamide 7. Yield: 35% (117 mg); mp 207–210 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 2.98–4.70 (m, 8H), 4.08 and 4.53 (ABq, 4H, J = 14.1 Hz), 6.64–

7.67 (m, 22H), 7.87 (br s, 2H); ^{13}C NMR: (100.4 MHz, $CDCl_3$) δ 38.7, 47.9, 66.5, 122.8, 128.1, 128.6, 129.0, 129.6, 130.2, 134.4, 177.9; IR (KBr, cm^{-1}): 3253, 1677, 1594; FAB MS (M^+): *m/z*: 668; Elemental Anal. calcd for $C_{40}H_{36}N_4O_6$: C, 71.84; H, 5.43; N, 8.38. Found: C, 71.75; H, 5.59; N, 8.33.

4.3.2. Cyclophane tetraamide 8. Yield: 40% (170 mg); mp 194–197 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 2.36 (s, 6H), 3.18–3.72 (m, 8H), 5.00 and 5.17(ABq, 4H, J = 13.2 Hz), 6.50–7.84 (m, 28H), 8.26 (br s, 2H); ^{13}C NMR: (100.4 MHz, $CDCl_3$) δ 19.8, 38.6, 47.9, 68.7, 111.8, 120.6, 127.1,127.4, 127.7, 127.8, 128.1, 129.1, 129.4, 129.7, 129.8, 130.2, 132.1, 135.9, 136.8, 140.4, 141.6, 154.1, 170.4; IR (KBr, cm^{-1}): 3253, 1655, 1593; FAB MS (M^+): *m/z*: 848; Elemental Anal. calcd for $C_{54}H_{48}N_4O_6$: C, 76.39; H, 5.70; N, 6.60. Found: C, 76.55; H, 5.59; N, 6.53.

4.3.3. Cyclophane tetraamide 9. Yield: 33% (135 mg); mp 260–263 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 3.28–4.02 (m, 8H), 5.01 and 5.15 (ABq, 4H, J = 12.4 Hz), 6.80–7.51 (m, 30H), 7.98 (br s, 2H); ^{13}C NMR: (100.4 MHz, $CDCl_3$) δ 39.6, 48.2, 70.0, 112.5, 121.0, 126.5, 127.1, 127.3, 127.5, 127.6, 127.9, 128.9, 129.7, 130.6, 136.1, 136.9, 1396.5, 142.4, 154.7, 170.4; IR (KBr, cm^{-1}): 3222, 1640, 1595; FAB MS (M^+): *m/z*: 820; Elemental Anal. calcd for $C_{52}H_{44}N_4O_6$: C, 76.08; H, 5.40; N, 6.82. Found: C, 76.25; H, 5.50; N, 6.53.

4.3.4. Cyclophane tetraamide 10. Yield: 37% (127 mg); mp 274–276 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 2.93–3.91 (m, 8H), 6.85–8.04 (m, 26H), 8.65 (br s, 2H); ^{13}C NMR: (100.4 MHz, $CDCl_3$) δ 39.9, 41.0, 48.2, 48.6, 106.1, 126.6, 126.8, 127.4, 127.8, 127.9, 128.6, 129.0, 129.5, 130.1, 130.3, 131.2, 131.7, 136.3, 142.8, 164.7, 170.4; IR (KBr, cm^{-1}): 3284, 1636, 1593; FAB MS (M^+): *m/z*: 684; Elemental Anal. calcd for $C_{44}H_{36}N_4O_4$: C, 77.17; H, 5.30; N, 8.18. Found: 77.25; H, 5.40; N, 8.53.

4.3.5. Cyclophane tetraamide 11. Yield: 35% (127 mg); mp 240–242 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 3.06–4.50 (m, 8H), 6.79–7.82 (m, 21H), 8.74 (br s, 2H); IR (KBr, cm^{-1}): 3058, 1640, 1593; FAB MS (M^+): *m/z*: 609; Elemental Anal. calcd for $C_{37}H_{31}N_5O_4$: C, 72.89; H, 5.13; N, 11.5. Found: C, 72.81; H, 5.16; N, 11.3.

4.4. General procedure for the preparation of cyclophane amides 13–16 by O-alkylation

A mixture of dichloro compound (0.5 mmol), dihydroxy compound (0.5 mmol), anhydrous potassium carbonate (2.0 mmol), two crystals of KI and acetonitrile (600 mL) was refluxed for 12 h. The reaction mixture was filtered and the solvent was evaporated under vacuum. The crude cyclophane amide was purified by column chromatography.

4.4.1. Cyclophane amide 13. Yield: 45% (47 mg); mp 220–222 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, DMSO-d_6) δ 4.76 (s, 4H), 7.04–7.63 (m, 8H), 9.71 (s, 2H); IR (KBr, cm^{-1}): 3058, 1640, 1593; MS (EI): *m/z*: 208; Elemental Anal. calcd for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.69; N, 9.39. Found: C, 64.48; H, 4.68; N, 9.38.

4.4.2. Cyclophane amide 14. Yield: 40% (68 mg); mp 218–220 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, DMSO-d_6) δ 2.58 (s, 3H), 5.01 (s, 4H), 6.47–7.81 (m, 7H), 10.72 (s, 2H); IR (KBr, cm^{-1}): 3023, 2787, 1740,1669, 1581; MS (EI): *m/z*: 340 Elemental Anal. calcd for $C_{18}H_{16}N_2O_5$: C, 63.52; H, 4.74; N, 8.23. Found: C, 63.54; H, 4.77; N, 8.22.

4.4.3. Cyclophane amide 15. Yield: 43% (73 mg); mp 208–210 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, DMSO-d_6) δ 2.54 (s, 3H), 4.33 (s, 2H), 5.11(s, 2H), 6.47–7.81 (m, 7H), 10.72 (s, 1H), 12.56 (s, 1H); ^{13}C NMR: (100.4 MHz, DMSO-d_6) δ 26.6, 65.7, 101.5, 107.5, 114.0, 116.3, 122.2, 123.5, 125.4, 133.1, 163.8, 164.2, 166.2; IR (KBr, cm^{-1}): 3020, 2777, 1745,1663, 1591; MS (EI): *m/z*: 340; Elemental Anal. calcd for $C_{18}H_{16}N_2O_5$: C, 63.52; H, 4.74; N, 8.23. Found: C, 63.55; H, 4.75; N, 8.46.

4.4.4. Cyclophane amide 16. Yield: 43% (73 mg); mp 214–216 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, DMSO-d_6) δ 2.55 (s, 3H), 4.34 (s, 2H), 5.10 (s, 2H), 6.48–7.64 (m, 7H), 10.72 (s, 1H), 11.89 (s, 1H); ^{13}C NMR: (100.4 MHz, DMSO-d_6) δ 22.0, 66.3, 95.4, 102.9, 109.9, 113.8,116.4, 122.2, 123.5, 125.5, 134.1, 158.0, 160.2, 166.4; IR (KBr, cm^{-1}): 3020, 2902, 1726,1672,1620,1542; MS (EI): *m/z*: 340; Elemental Anal. calcd for $C_{18}H_{16}N_2O_5$: C, 63.52; H, 4.74; N, 8.23. Found: C, 63.54; H, 4.73; N, 8.42.

4.5. General procedure for the preparation of cyclophane amides 17–18 by N-alkylation

A mixture of dihalo compound (0.5 mmol), diamide **6** (0.5 mmol), anhydrous potassium carbonate (2.0 mmol), two crystals of KI and acetonitrile (600 mL) was refluxed for 24 h. The reaction mixture was filtered and the solvent was evaporated under vacuum. The crude cyclophane amide was purified by column chromatography.

4.5.1. Cyclophane amide 17. Yield: 35% (107 mg); mp 230–232 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^{1}H NMR: (400 MHz, $CDCl_3$) δ 2.15 (s, 6H), 3.11–3.89 (m, 8H), 4.49 (s, 4H), 6.70–7.41 (m, 20H), 8.04 (br s, 2H); ^{13}C NMR: (100.4 MHz, $CDCl_3$) δ 19.5, 38.9, 49.8, 51.3, 112.8, 117.2, 126.9, 127.6, 127.9, 128.3, 128.6, 129.3, 129.6, 132.9, 133.2, 135.2, 139.6, 145.0, 148.7, 170.0; IR (KBr, cm^{-1}): 3311, 1636, 1578; FAB MS: *m/z*: 608; Elemental Anal. calcd for $C_{40}H_{40}N_4O_2$: C, 78.92; H, 6.62; N, 9.20. Found: C, 78.68; H, 6.48; N, 9.41.

4.5.2. Cyclophane amide 18. Yield: 40% (107 mg); mp 188–190 °C; eluent for column chromatography: $CHCl_3$/MeOH (99:1); ^1H NMR: (400 MHz, $CDCl_3$) δ 3.23–3.47 (m, 8H), 4.45 (s, 4H), 6.68–7.64 (m, 30H), 8.24 (br s, 2H); ^{13}C NMR: (100.4 MHz, $CDCl_3$) δ 37.0, 49.7, 53.3, 112.4, 112.5, 125.8, 126.6, 127.0, 127.3, 127.7, 129.0, 129.2, 129.3, 129.4, 139.7, 141.2, 170.3; IR (KBr, cm^{-1}): 3247, 1640, 1597, 1503. FAB MS: *m/z*: 732; Elemental Anal. calcd for $C_{50}H_{44}N_4O_2$: C, 81.94; H, 6.05; N, 7.65. Found: C, 81.68; H, 6.18; N, 7.41.

4.6. General procedure for the synthesize of bicyclic hexaamide 21

A solution of the 1,3,5-benzene tricarboxylic acid chloride (0.5 mmol) in dry chloroform (100 mL) and a solution of the diamine (0.5 mmol) and triethyl amine (1.6 mmol) in dry chloroform (100 mL) were simultaneously added dropwise during 6 h to a well-stirred solution of chloroform (500 mL) at reflux. After the addition was complete, the reaction mixture was refluxed for another 6 h. The solvent was removed at reduced pressure and the residue obtained was then dissolved in chloroform (300 mL), washed with water (2× 100 mL) to remove the triethylammonium chloride and dried over magnesium sulfate. Removal of the chloroform gave the cyclophane as a crude material, which was purified by column chromatography (SiO_2).

4.6.1. Bicyclic hexaamide 21. Yield: 15% (54 mg); mp >300 °C; eluent for column chromatography: $CHCl_3$/MeOH (19:1); IR (KBr, cm^{-1}): 3324, 1665, 1598; FAB MS: *m/z*: 720; Elemental Anal. calcd for $C_{42}H_{36}N_6O_6$: C, 69.99; H, 5.03; N, 11.6. Found: C, 69.91; H, 5.06; N, 11.4.

4.7. General procedure for the synthesize of compound 22

To a solution of KOH (19.7 mmol) in methanol (40 mL) was added 2-aminothiophenol (19.2 mmol) followed by monohalide (15.0 mmol) at 30 °C with stirring. After stirring for 4 h, the solid obtained was filtered with suction, washed with methanol (25 mL) and then with water (50 mL) and dried with suction to give pure compound **22**.

4.7.1. Compound 22. Yield: 80% (3.1 g); mp 178–180 °C; ^1H NMR: (90 MHz, $CDCl_3$) δ 3.60 (s, 2H), 4.60 (br s, 2H) 6.40 7.60 (m, 9H), 9.20 (br s, 1H); IR (KBr, cm^{-1}): 3332, 2934, 1664, 1521; MS (EI): *m/z*: 258; Elemental Anal. calcd for $C_{14}H_{14}N_2OS$: C, 65.09; H, 5.46; N, 10.8. Found: C, 65.27; H, 5.31; N, 10.7.

4.8. General procedure for the synthesize of diamine 23

To a mixture of compound **22** (1 g, 4.0 mmol) and sodium borohydride (10 g, excess) in THF (50 mL) was added glacial acetic acid (6 mL) at reflux for 6 h. After 6 h of reflux, the reaction mixture was cooled and quenched with ice water (200 mL). The organic layer was extracted with ether (4× 25 mL). The ether extracts were washed with water (2× 25 mL) and dried over anhydrous sodium sulfate. After removing ether, the residue was chromatographed over SiO_2 to give diamine **23** as a clear liquid.

4.8.1. Diamine 23. Yield: 60% (3.1 g); eluent for column chromatography: hexane/$CHCl_3$ (1:1); ^1H NMR: (90 MHz, $CDCl_3$) δ 3.00 (t, 2H, $J = 4.2$ Hz), 3.30 (m, 2H), 4.10 (br s, 3H), 6.30–7.40 (m, 9H); IR (KBr, cm^{-1}): 3332, 2934, 1664, 1521; MS (EI): *m/z*: 244; Elemental Anal. calcd for $C_{14}H_{16}N_2S$: C, 68.81; H, 6.60; N, 11.4. Found: C, 68.77; H, 6.41; N, 11.5.

4.9. Anti-bacterial assay method

The disc diffusion assay was used to determine the antibacterial activity of the test compounds using *E. coli*, *P. vulgaris* (Gram negative) and *S. aureus*, *St. faecalis* (Gram positive) as the test bacteria. Various concentrations of cyclophane amides **7**–**9**, **13**, **15**–**18** and **21** were prepared by dissolving 50 μg, 100 μg and 150 μg in 1 mL of dimethylsulfoxide (DMSO). The synthetic compounds were loaded into the filter paper disc in different concentration [50 μg, 100 μg, 150 μg]; the discs were air-dried. Base plates were prepared by pouring 10 mL of Nutrient agar (NA) into the sterile Petri plates and allowed to set. The bacteria were subcultured in nutrient broth from which 100 μL of cell suspension was taken and its OD was adjusted to 0.5, after which this was spread as a thin film over the nutrient agar plates. Then the loaded filter paper discs were placed on the surface of nutrient agar plates. The plates were incubated at 37 °C for 24 h and inhibition zone was thus measured. DMSO (150 μL) loaded filter paper disc was used as negative control. The standard antibiotic disc (streptomycin 10 μg/disc) was used as positive control.

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