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Synthesis, complexation studies and biological activity of some novel mono and tricyclic cyclophane amides and cyclophane sulfonamides

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ABSTRACT

A series of mono, tricyclic cyclophane tetraamides and cyclophane sulfonamides have been synthesized and characterized from spectral and XRD studies. All the cyclophane amides form charge transfer (CT) complex with TCNQ. The cyclophane amides show moderate to good anti-inflammatory activity. Some of them were active against Gram positive (*Klebsiella pneumonia*) and Gram negative (*Escherichia coli* and *Staphylococcus aureus*) human pathogens.

1. Introduction

The development of synthetic receptors for anions is of considerable interest because of their potential biomedical and environmental applications.[1] The discovery of crown ethers led the breakthrough in the field of molecular recognition.[2] Synthesis of aza-crown ether based cyclophanes with amide linkage would be of greater interest due to their potential applications in the field of material science, medicine, biology[3] and such molecules strongly bind with alkali metal ions also. The enhanced applications of aza crown ethers in supramolecular chemistry[4] have influenced the synthetic chemist to modify the molecular structure. The most important aspect of supramolecular chemistry is the host–guest complexation process. Hence the structures of basic crown ethers were modified significantly to increase the selectivity and specificity of guest molecules for complexation. One of the key modification is the replacement of oxygen donor atoms by sulfur and/or by nitrogen atoms.[5] The other significant modification is the insertion of functional groups viz., amides and esters in the macrocyclic ring system.[6]

Synthesis of mixed aza-oxo-thia macrocyclic tetraamides and their antimicrobial, cytotoxicity studies were recently reported.[7] Cyclic peptides with fluorophore-tag are useful for the design of Hg^{2+} sensors.[8] Synthesis of amide-based supramolecular systems has been reported in the literature.[9,10] Supramolecular amides are also used as molecular clefts,[11] molecular receptors,[12] and in molecular recognition[13] of biologically interacting substrates including anti-HIV active macrocyclic amides.[14] The chiral macrocyclic tetraamides and their transition metal complexes have also been reported.[15] Interaction between an electron donor and an electron acceptor moiety in cyclophane amides could result in the formation of either a charge transfer (CT) complex or π–π stacking interactions.[16–19] Such cyclophane amides are in general called as self-complementary cyclophane amides. Developing cyclophane amide systems, which exhibit CT complexation, metal complexation,[20,21] and biological activity[22] is an ever interesting and challenging problem. Due to their structural versatility and opportunity for synthetic modifications, amide cyclophanes incorporating phenyl[23] and *m*-terphenyl unit have received much attention in the area of CT complexation, ion transportation studies, and biological activity.[24] Hence, it is of interest to synthesize and study the CT complexation, metal complexation properties, and biological activity of novel cyclophane amides. Recently, synthesis of dicationic acridinophanes,[25] quinolinophanes,[26] carbazolophanes,[27] and imidazolophanes[28] using various spacers like pyridine, *m*-terphenyl, and chiral binaphthol has been reported from our laboratory. Herein, we report the synthesis of monocyclic and tricyclic cyclophane amides, sulfonamides **1**–**13** (Figs. 1 and 2) and

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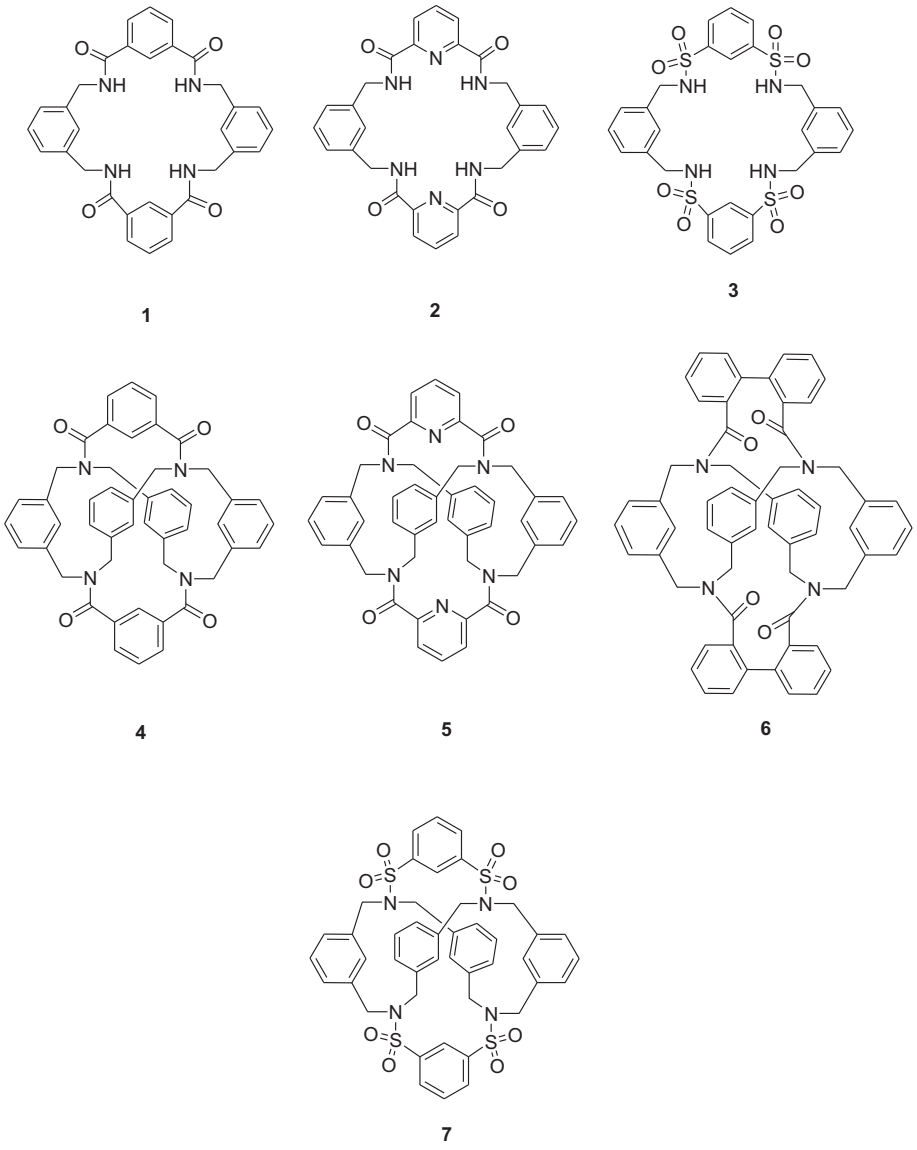


Fig. 1. Structure of cyclophane amides and sulfonamides **1**–**7**.

their charge transfer complexation studies with 7,7,8,8-tetracyanoquinodimethane (TCNQ). We also wish to report the preliminary biological evaluation of all the synthesized compounds for their anti-inflammatory and anti-bacterial activities.

2. Results and discussion

2.1. Chemistry

The synthetic pathway leading to monocyclic cyclophane amides **1**, **2** and sulfonamide **3** (Fig. 1) is outlined in Scheme 1. Reaction of 1.0 equiv of *m*-xylylene diamine with 1.0 equiv of each isophthaloyl chloride, pyridine-2,6-dicarboxylic acid chloride, and benzene-1,3-disulfonyl chloride in presence of triethylamine in dry dichloromethane at room temperature under high dilution conditions afforded the cyclophane amides **1**, **2** and sulfonamide **3** in about 80, 80, and 60% yields, respectively (Scheme 1). The ^{1}H NMR spectrum of cyclophane sulfonamide **3** displayed the *N*-methylene protons as a doublet at δ 4.21 and NH protons as a triplet at δ 8.37. The rest of the aromatic protons appeared between δ 6.83 and 8.07. In the ^{13}C NMR spectrum of **3**, the *N*-methylene carbons appeared at δ 47.1. The FTIR spectrum of **3** showed the sulfonyl stretching frequency at 1631 cm^{-1} and the mass spectrum showed the molecular ion peak ([M+H]$^{+}$) at *m*/*z* 677.6. Similarly the structure of cyclophane amides **1** and **2** was confirmed from spectral and analytical data. All the new compounds gave satisfactory FTIR, ^{1}H and ^{13}C NMR, mass spectral, and elemental analysis.

In order to test the synthetic utility of tetraamine cyclophane **14** for the synthesis of tricyclic cyclophane amides, 1.0 equiv of cyclophane amine **14** was coupled with 2.0 equiv of each isophthaloyl chloride, pyridine-2,6-dicarboxylic acid chloride, diphenic acid chloride, and benzene-1,3-disulfonyl chloride in presence of triethylamine in dry DCM at room temperature under high dilution conditions. The reaction mixture after usual work-up afforded the tricyclic cyclophane amides **4**, **5**, **6**, and **7** in 65, 70, 50, and 75% yields, respectively, after purification by column chromatography (Scheme 2). The ^{1}H NMR spectrum of cyclophane amide **5** displayed four sets of doublets for the *N*-methylene protons at δ 3.80, 5.00, 5.10, and 5.41. The rest of the aromatic protons appeared between δ 6.95 and 7.94. In the ^{13}C NMR spectrum of cyclophane amide **5**, the *N*-methylene carbons appeared at δ 50.8 and 55.0 and carbonyl carbon at δ 169.2. The FTIR spectrum of **5**

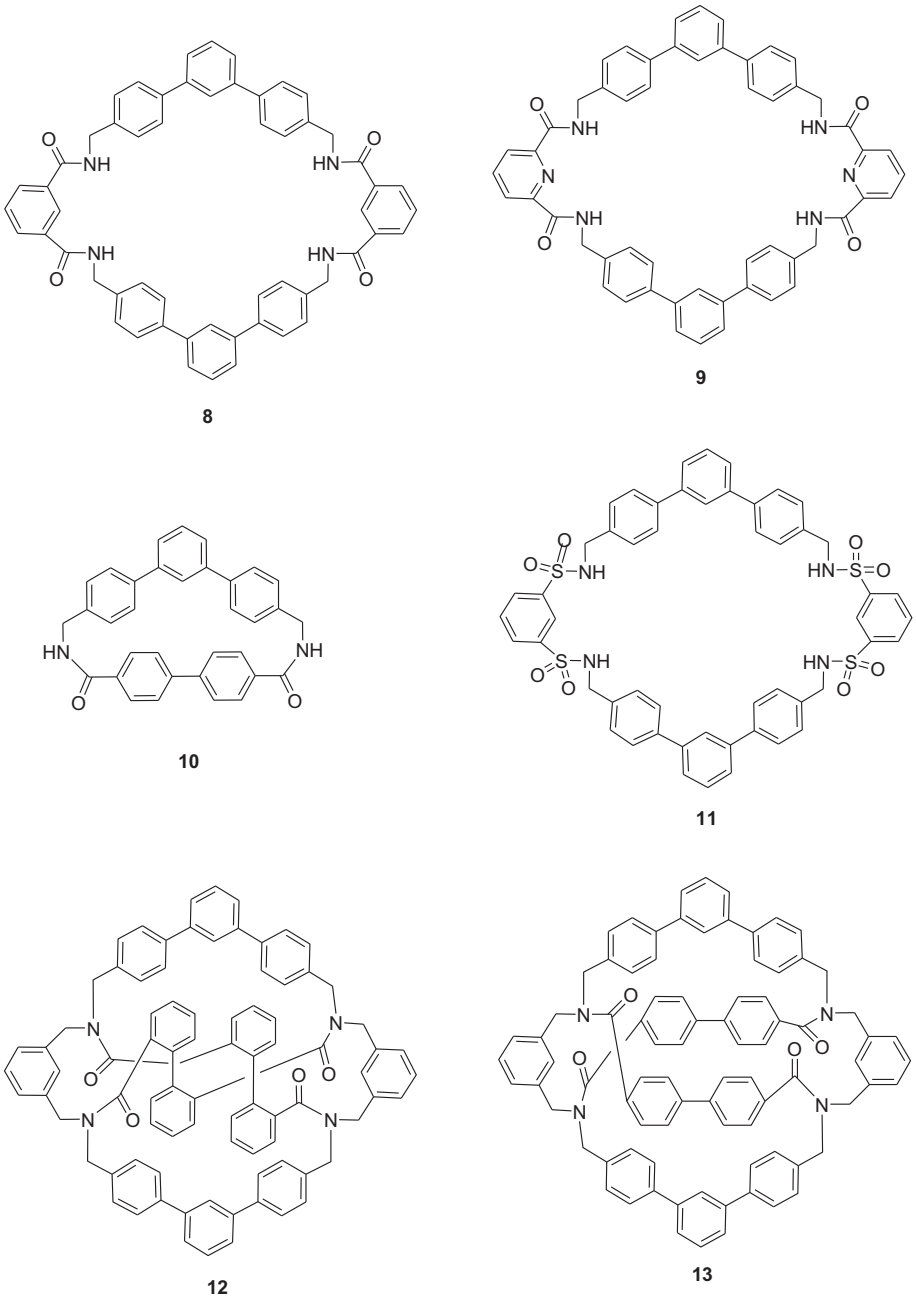
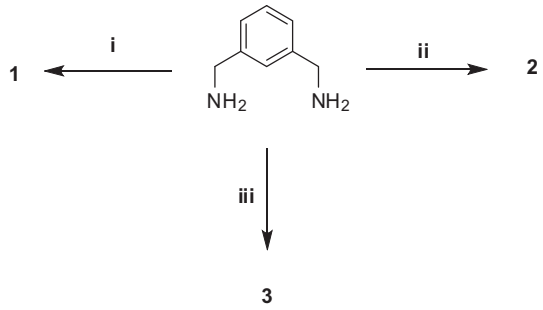


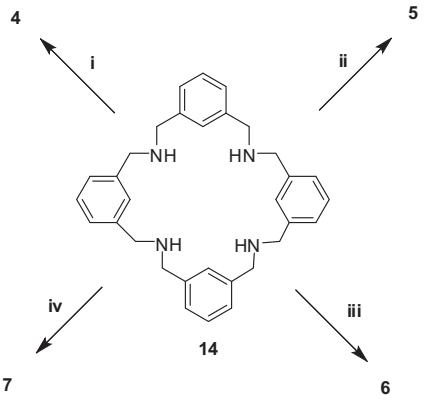
Fig. 2. Structure of cyclophane amides and sulfonamides **8**–**13**.

showed the carbonyl stretching frequency at 1631 cm^{-1} and the mass spectrum showed the molecular ion peak ($[M+H]^+$) at *m*/*z* 739.3. The tetraamide cyclophane **5** gave satisfactory elemental analysis. The amide **5** was recrystallized from chloroform/acetonitrile mixture. XRD studies of compound **5** show that out of four benzene rings, two of them are parallel to each other. Moreover, the pyridine moieties are parallel to each other. XRD studies indicate that intermolecular hydrogen bonding exists between cyclophane amide **5** and water. The crystal parameters for cyclophane amide **5** are given in Table 1 in Supplementary data and ORTEP diagram is shown in Fig. 3. Similarly the structure of the tricyclic amide cyclophanes **4**, **6** and sulfonamide **7** was also confirmed from spectral and analytical data.

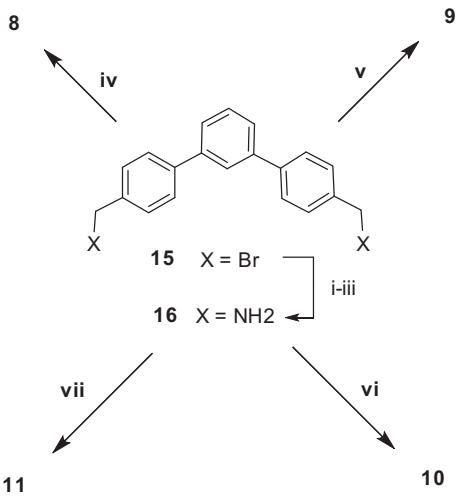
Electron rich cyclophanes with large cavities[29] are known to bind electron-deficient guest molecules effectively. Hence, we focused our attention on the synthesis of monocyclic cyclophane amides, sulfonamide with *m*-terphenyl spacer. The synthetic pathway leading to monocyclic cyclophane amides **8**, **9**, **10** and sulfonamide **11** (Fig. 2) is outlined in Scheme 3. In order to synthesize cyclophane amides with large cavity size to study the influence of cavity size on CT complexation and biological studies, diamine **16** was prepared. Reaction of 1.0 equiv of *m*-terphenyl dibromide **15** with 2.2 equiv of hexamine in dry chloroform at reflux resulted in the formation of hexammonium salt, which on further hydrolysis with hydrochloric acid in EtOH/H_2O mixture at reflux afforded diamine **16** in 90% yield. The ^1H NMR spectrum of diamine **16** displayed the *N*-methylene protons as a singlet at δ 3.93. The rest of the aromatic protons appeared in the region δ 7.40–7.79. In the ^{13}C NMR spectrum of **16**, the *N*-methylene carbons appeared at δ 45.4 and the mass spectrum showed the molecular ion peak ($[M+H]^+$) at *m*/*z* 289.2. Reaction of 1.0 equiv of *m*-terphenyl diamine **16** with 1.0 equiv of each isophthaloyl



Scheme 1. Reagents and conditions: (i) isophthaloyl chloride, TEA, DCM, rt, 24 h, **1** (80%); (ii) pyridine-2,6-dicarboxylic acid chloride, TEA, DCM, rt, 24 h, **2** (80%); (iii) benzene-1,3-disulfonyl chloride, TEA, DCM (dry), 24 h, **3** (60%).



Scheme 2. Reagents and conditions: (i) isophthaloyl chloride, TEA, DCM, rt, 24 h, **4** (65%); (ii) pyridine-2,6-dicarboxylic acid chloride, TEA, DCM, rt, 24 h, **5** (70%); (iii) diphenic acid chloride, TEA, DCM, rt, 24 h, **6** (50%); (iv) benzene-1,3-disulfonyl chloride, TEA, DCM, rt, 24 h, 75%.



Scheme 3. Reagents and conditions: (i) hexamine, $CHCl_3$, rt, 12 h; (ii) concd HCl, EtOH/H_2O, reflux, 3 h; (iii) NaOH/H_2O, rt, **22** (90%); (iv) isophthaloyl chloride, TEA, DCM (dry), 24 h, **8** (70%); (v) pyridine-2,6-dicarboxylic acid chloride, TEA, DCM (dry), 24 h, **9** (80%); (vi) biphenyl-4,4′-dicarboxylic acid chloride, TEA, DCM (dry), 24 h, **10** (80%); (vii) benzene-1,3-disulfonyl chloride, TEA, DCM (dry), 24 h, **11** (70%).

chloride, pyridine-2,6-dicarboxylic acid chloride, biphenyl-4,4′-dicarboxylic acid chloride, and benzene-1,3-disulfonyl chloride in presence of triethylamine in dry dichloromethane at room temperature under high dilution conditions afforded the cyclophane amides, **8**, **9**, **10**, and **11** in about 70, 80, 80, and 70% yields, respectively (Scheme 3). The ^{1}H NMR spectrum of cyclophane amide **8** displayed the *N*-methylene protons as a broad singlet at δ 4.54 and NH protons as a multiplet between δ 9.19 and 9.24. The rest of the aromatic protons appeared in the region δ 7.43–8.49. In the ^{13}C NMR spectrum of **8**, the *N*-methylene carbons appeared

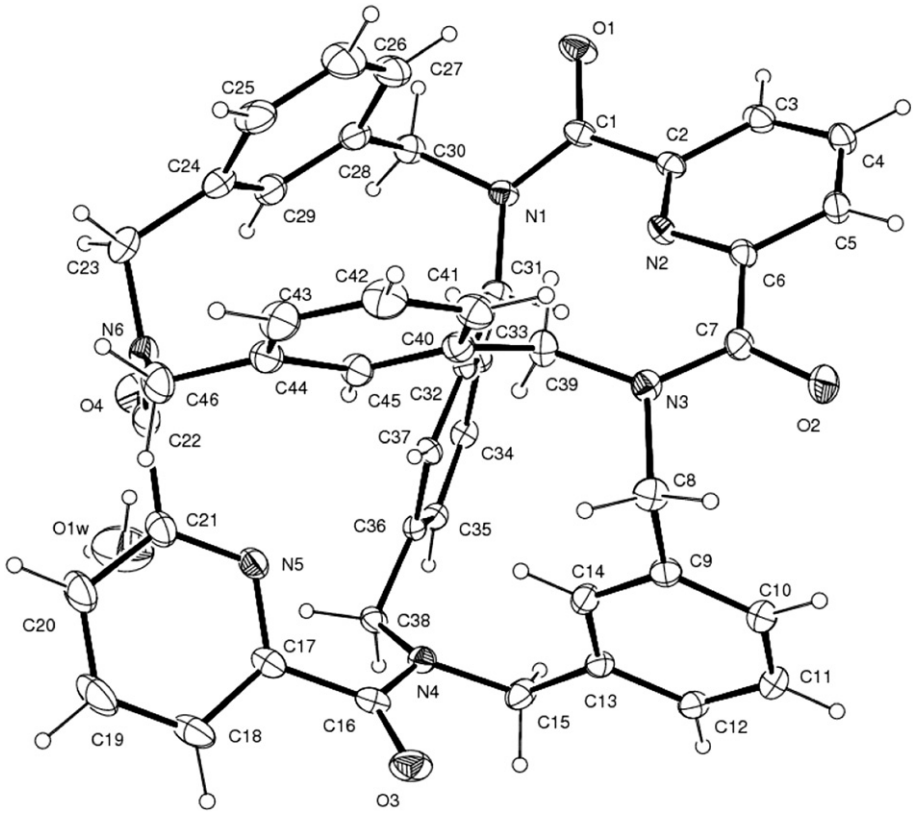
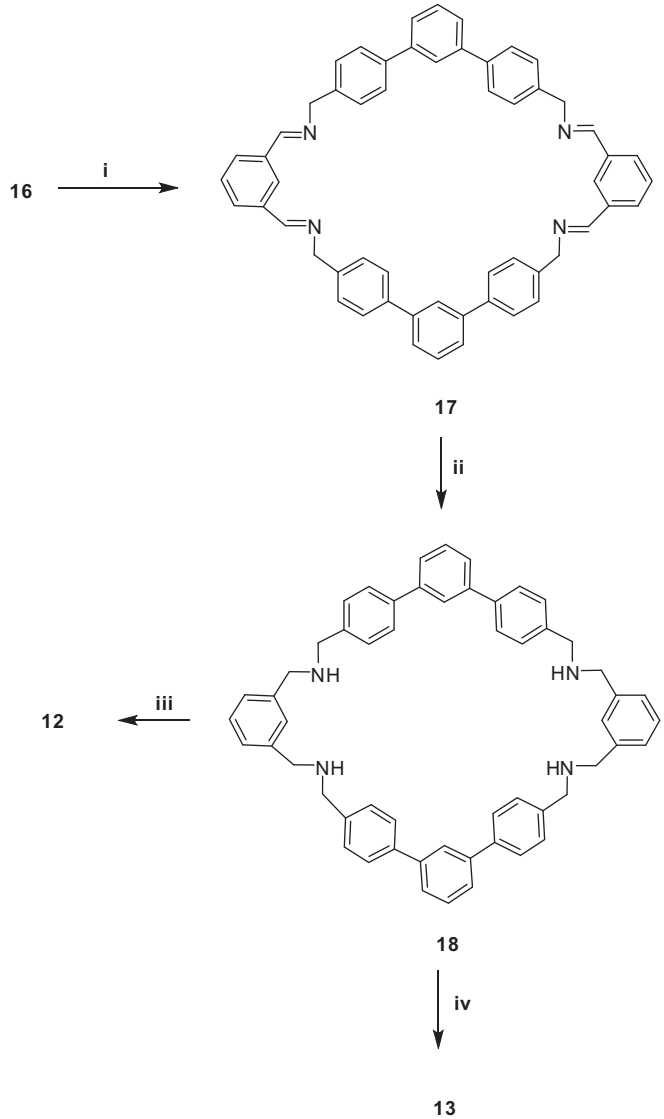


Fig. 3. ORTEP diagram of cyclophane amide **5**.

at δ 42.5 and the carbonyl carbon at δ 165.6 and 165.9. The FTIR spectrum of **8** showed the carbonyl stretching frequency at 1641 cm^{-1} and the mass spectrum showed the molecular ion peak ($[M-H]^-$) at *m*/*z* 835.6. Similarly the structure of the amide cyclophanes **9**, **10** and sulfonamide cyclophane **11** was also confirmed from spectral and analytical data.

In order to synthesis tricyclic cyclophane tetraamides **12** and **13**, the diamine **16** was used. Reaction of 1.0 equiv of *m*-terphenyl diamine **16** and 1.0 equiv of isophthalaldehyde in ethanol under high dilution conditions at room temperature resulted in the formation of cyclophane tetraimine **17** in 90% yield (Scheme 4). The product slowly precipitated from the reaction mixture during the course of the reaction and was used without further purification. The ^1H NMR spectrum of tetraimine cyclophane **17** displayed the *N*-methylene protons as a singlet at δ 4.85. The *N*=*CH* protons appeared as a singlet at δ 8.45 and the aromatic protons appeared between δ 7.39 and 7.85. In the ^{13}C NMR spectrum of **17**, the *N*-methylene carbons appeared and *N*=*CH* carbons at δ 64.9 and 161.6, respectively, and the mass spectrum showed the molecular ion peak ($[M+H]^+$) at *m*/*z* 773.4.



Scheme 4. Reagents and conditions: (i) isophthalaldehyde, ethanol, rt, 48 h (90%); (ii) $NaBH_4$, toluene/THF/MeOH, 0–5 °C, 1 h, **18** (80%); (iii) diphenic acid chloride, TEA, DCM, rt, 24 h, **12** (45%); (iv) biphenyl-4,4′-dicarboxylic acid chloride, TEA, DCM, rt, 24 h, **13** (50%).

Reduction of cyclophane imine **17** with sodium borohydride in toluene/THF/MeOH mixture at 0–5 °C afforded secondary tetraamine cyclophane **18** in about 80% yield (Scheme 4). The ^1H NMR spectrum of tetraamine cyclophane **18** displayed the *N*-methylene protons as a singlet at δ 3.85 and the aromatic protons appeared between δ 7.27 and 7.71. In the ^{13}C NMR spectrum of **18**, the *N*-methylene carbons appeared at δ 51.7 and 52.1 and the mass spectrum showed the molecular ion peak ($[M+H]^+$) at *m*/*z* 781.5.

Cyclophane tetraamine **18** of 1.0 equiv was coupled with 2.2 equiv of diphenic acid chloride and biphenyl-4,4′-dicarboxylic acid chloride in presence of triethylamine in dry dichloromethane at room temperature under high dilution conditions. The reaction afforded the tricyclic cyclophane amides **12** and **13** in 45 and 50% yields, respectively (Scheme 4). The ^1H NMR spectrum of cyclophane amide **12** displayed *N*-methylene protons as a pair of doublets at δ 3.80, 3.84, a quartet at δ 4.62, and a pair of doublets at δ 4.83 and 4.87. The rest of the aromatic protons appeared between δ 6.71 and 8.31. In the ^{13}C NMR spectrum of cyclophane amide **12**, the *N*-methylene carbons appeared at δ 49.0 and 52.5 and carbonyl carbon at δ 170.7. The FTIR spectrum of **12** showed the carbonyl stretching frequency at 1634 cm^{-1} and the mass spectrum showed the molecular ion peak ($[M+H]^+$) at *m*/*z* 1193.7. Similarly the structure of tricyclic cyclophane amide **13** was confirmed from spectral and analytical data.

2.2. Complexation studies

Cyclophane amides **1**–**13** form charge transfer complexes with 7,7,8,8-tetracyanoquinodimethane (TCNQ).[30] Complexation studies of compounds **1**–**13** with tetracyanoethylene (TCNE) and paraquat (PQT) were not successful. Cyclophanes **1**–**13** show UV–vis absorption maxima at 255.0, 258.0, 238.5, 225.5, 261.0, 221.5, 262.0, 255.0, 258.0, 270.5, 241.5, 255.5, and 257.5 nm, respectively. However, the acceptor TCNQ shows an absorption maximum at 395.0 nm. Cyclophanes, **1**–**13** form a charge transfer complex with TCNQ as evidenced by the appearance of absorption maxima at 843.0, 842.5, 742.5, 843.5, 842.0, 742.0, 842.5, 743.5, 742.5, 742.5, 743.0, 743.0, and 743.5 nm, respectively (Table 1 and Fig. 4). The studies were carried out as outlined below.

A solution of TCNQ (4.9×10^{-6} M) in a 1:1 mixture of $CHCl_3$/CH_3CN at various dilutions (1, 2, 3, 4, 5, and 6 mL) were prepared and added to the solution of the cyclophane amide (2.34×10^{-5} M) in a 1:1 mixture of $CHCl_3$/CH_3CN (3 mL) in a quartz cuvette of path length 1 cm. The UV–vis spectrum was also obtained for each of the sample separately and the changes in the absorbance of CT bands were recorded. The CT complexation study of **6** with various concentrations of TCNQ is shown in Table 2. The plot of (concentration of cyclophane)/absorbance (*Y*/*A*) vs 1/concentration of guest (1/*X*)

Table 1
λ_{max} for cyclophane amides and TCNQ complex of cyclophane amides **1**–**13**

Cyclophane amide	λ_{max} (nm) of cyclophane amide	λ_{max} (nm) of TCNQ complex
1	255.0	843.0
2	258.0	842.5
3	238.5	742.5
4	225.5	843.5
5	261.0	842.0
6	221.5	742.0
7	262.0	842.5
8	255.0	743.5
9	258.0	742.5
10	270.5	742.5
11	241.5	743.0
12	255.5	743.0
13	257.5	743.5

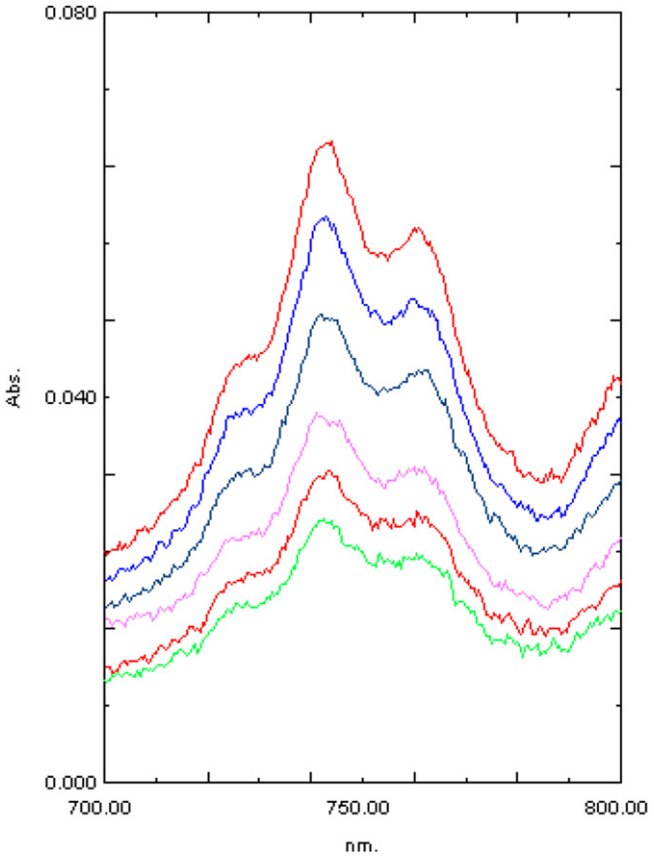


Fig. 4. Charge transfer complexation behavior of cyclophane amide **6** with variable concentration of TCNQ.

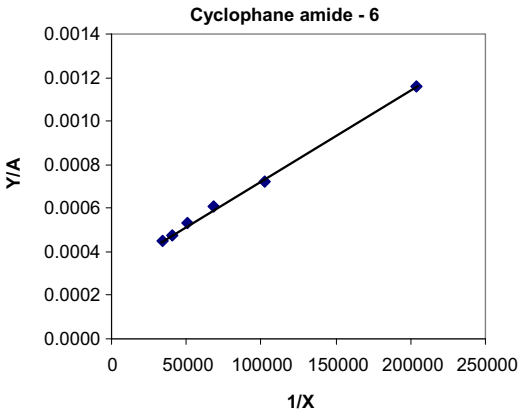


Fig. 5. Plot between $1/X$ and Y/A for cyclophane amide **6**.

was linear (Fig. 5). Benesi–Hildebrand equation was employed to calculate K_a values.[30] From the slope and the intercept values, K_a (K_a=intercept×slope^{-1}) and ε(ε=intercept^{-1}) were evaluated. The plot was linear suggesting that the predominate species in solution as a 1:1 complex (Fig. 5).

The K_a, ε, and r values of the CT complexes formed from **1** to **13** with TCNQ are shown in Table 3. All the compounds showed above effectively forms charge transfer complexes with TCNQ. Cyclophane amide (relatively smaller cavity) **3**, **6**, and **4** bind TCNQ more strongly than **5**, **2**, **7**, and **1**. However, compounds (larger cavity) **11**, **13**, and **9** bind TCNQ more strongly than **8**, **12**, and **10**. The nitrogen atoms of the pyridine ring in the cyclophane amides moderately influence the binding ability. The sulfonyl groups at the annular ring in the cyclophane amides also greatly influence the binding ability. But the influence of other substituents like benzene and biphenyl toward the binding ability is relatively less when compared with other functional groups. Complexation studies of **1**–**13** with TCNE and PQT were not successful.

Table 2
Benesi–Hildebrand treatment data of the CT complex formed between the cyclophane amide, **6** and TCNQ

Concn of guest, [X] (M)	Absorbance, A	[Y]/A (M)	1/[X] (M^{-1})
4.9×10^{-6}	0.023	0.001161	204,081
9.8×10^{-6}	0.037	0.000722	102,040
14.7×10^{-6}	0.044	0.000607	68,027
19.6×10^{-6}	0.050	0.000534	51,020
24.5×10^{-6}	0.056	0.000477	40,816
29.4×10^{-6}	0.059	0.000453	34,013

λ_{max}=742.0 nm; concentration of cyclophane amide, **6**=2.67×10^{-5} M.
K_a=7.60×10^4 M^{-1}; ε=3.18×10^3 [M^{-1} cm^{-1}] and r=0.9993.

Table 3
Complexation of TCNQ with cyclophane amides **1**–**13**

Cyclophane amide	K_a (mol^{-1} dm^3)	ε [M^{-1} cm^{-1}]	r
1	6.34×10^3	2.48×10^4	0.9997
2	1.22×10^4	1.68×10^4	0.9981
3	8.06×10^4	5.96×10^3	0.9985
4	3.79×10^4	1.80×10^4	0.9991
5	1.57×10^4	2.03×10^4	0.9996
6	7.60×10^4	3.18×10^3	0.9993
7	1.11×10^4	2.34×10^4	0.9999
8	8.75×10^4	5.16×10^3	0.9998
9	1.03×10^5	4.46×10^3	0.9998
10	4.64×10^3	4.51×10^3	0.9999
11	1.49×10^5	4.77×10^3	0.9999
12	6.65×10^4	6.87×10^3	0.9984
13	1.32×10^5	2.54×10^3	0.9991

2.3. Biological study

In vitro anti-inflammatory activity was studied by HRBC (human red blood cells) membrane stabilization method.[31] The lysosomal enzymes released during inflammatory condition produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The anti-inflammatory agent acts by either inhibiting the lysosomal enzymes or by stabilizing the lysosomal membranes. The observed data showing the anti-inflammatory activity of the compounds and the control drug are given in Table 4. In the present study the anti-inflammatory activity of these compounds was investigated using HRBC membrane stabilization and prednisolone was used as a standard and the results are tabulated (Table 4). The anti-inflammatory activities of all the amide cyclophanes **1**–**13** are concentration dependent. At higher concentration, the amide cyclophanes exhibit better anti-inflammatory activity than at lower concentration. Compound **8** shows the maximum anti-inflammatory activity (89.56% at 400 μg/mL) when compared to the reference drug prednisolone (95.24% at 400 μg/mL).

The highest degree of anti-inflammatory activity of cyclophane amides **1**–**13** were found to be 72.79, 68.96, 86.56, 74.70, 77.80, 84.30, 76.74, 89.56, 78.05, 62.66, 83.44, 83.77 and 79.31%, respectively, at 400 μg/mL (Table 4 and Fig. 6).

The anti-bacterial studies were carried out aseptically under in vitro conditions by 'cup plate method'.[32] All the five cyclophane amides exhibited different levels of anti-bacterial activity against the four tested human pathogenic bacteria compared to DMSO as control. The antimicrobial activities of five different newly synthesized cyclophane amides were evaluated against four human pathogenic bacteria such as, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. The biological

Table 4
In vitro anti-inflammatory activity of cyclophane amides **1**–**13** by HRBC membrane stabilization

Cyclophane amide	Activity (% prevention of lysis)			
	50 μg/mL	100 μg/mL	200 μg/mL	400 μg/mL
1	65.94±0.36	68.34±0.61	70.94±0.50	72.79±0.34
2	61.56±0.27	63.57±0.43	67.68±0.31	68.96±0.22
3	57.93±0.16	63.12±0.49	71.57±0.19	86.56±0.12
4	68.56±0.24	71.78±0.33	73.84±0.49	74.70±0.10
5	72.48±0.42	73.26±0.13	74.89±0.29	77.80±0.28
6	74.25±0.25	76.64±0.23	77.93±0.16	84.30±0.38
7	37.48±0.42	58.85±0.31	71.28±0.34	76.74±0.15
8	59.42±0.89	75.30±0.29	86.62±0.21	89.56±0.42
9	69.43±0.23	74.09±0.56	77.60±0.22	78.05±0.15
10	47.73±0.33	48.53±0.21	56.86±0.39	62.66±0.27
11	73.35±0.52	76.22±0.47	80.84±0.97	83.44±0.57
12	38.83±0.14	66.90±0.54	79.16±0.31	83.77±0.24
13	62.29±0.16	69.48±0.31	73.44±0.41	79.31±0.59
Prednisolone	57.94±0.39	84.87±0.36	91.01±0.45	95.24±0.21

Each value represents mean±SD of three observations.

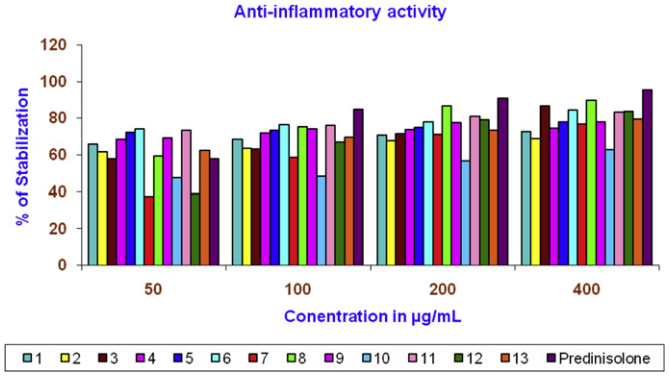


Fig. 6. In vitro anti-inflammatory activity of cyclophane amides **1**–**13**.

screening results of cyclophane amides with 10% DMSO as control and with commercial antibiotics viz. Gentamycin for *E. coli*, *S. aureus*, Ciprofloxacin for *K. pneumonia* and Cefotaxime for *P. aeruginosa* are tabulated (Table 5) in the form of diameter of zone of inhibition in mm.

Further the anti-bacterial activity of the test compound was dose dependent and it was remarkable at higher concentration. The diameter of the zone of inhibition of amides **1**–**7** against bacterial human pathogens is determined by the cup plate method between 250 and 2000 μg/mL. The cyclophane amides **4** and **5** show moderate activity against *S. aureus*. However, cyclophane amides **1**–**7** did not show any activity against *P. aeruginosa*. Cyclophane amides **6** and **7** showed remarkable activity against *K. pneumonia* species whereas compound **3** showed moderate activity. However, cyclophane amides **1** and **2** show activity in the range of 11.0–12.0 against *E. coli* (Table 5).

3. Conclusion

In conclusion, we have synthesized various monocyclic and tricyclic cyclophane amides, sulfonamides with small and large cavities. Charge transfer complexation studies of all the cyclophane amides were carried out with TCNQ. Cyclophane amide with relatively large cavity binds TCNQ more strongly than cyclophanes with smaller cavity. Complexation studies of **1**–**13** with TCNE and PQT were not successful. Some of the synthesized cyclophane amides were active against Gram positive (*K. pneumonia*) and Gram negative (*E. coli* and *S. aureus*) human pathogens. But they are not active against Gram positive human pathogen *P. aeruginosa*.

4. Experimental

4.1. General

All the reagents and solvents employed were of the best grade available and were used without further purification. The melting points were determined by using a Metler Toledo melting point apparatus by open capillary tube method and were uncorrected. Spectroscopic data were recorded by the following instruments: UV/vis: Shimadzu 2550 spectrophotometer. IR: Perkin–Elmer series 2000 FTIR spectrophotometer. NMR: Bruker Avance 400 MHz. Mass: ESI—PerkinElmer Sciex, API 3000 mass spectrometer and FAB-mass spectra: Jeol SX 102/DA-6000 mass spectrometer. The elemental analysis for the compounds was carried out using the Elementar Vario EL III elemental analyzer (SIPRA LABS LTD., Hyderabad, India). Pre-coated silica gel plates from Merck were used for TLC. Column chromatography was carried out using silica gel (100–200 mesh) purchased from ACME.

4.2. Procedure for the preparation of tetraimine

A solution of diamine (4.33 g, 15 mmol) in ethanol (400 mL) and a solution of dialdehyde (2.02 g, 15 mmol) in ethanol (400 mL) were simultaneously added dropwise to a well stirred solution of ethanol (800 mL) for 6–8 h. After the addition was complete, the reaction mixture was stirred for another 48 h. The precipitated solid was filtered, washed with ethanol, and dried.

*4.2.1. Tetraimine **17**.* This product was obtained as off-white solid (5.22 g, 90%). Found: C, 87.29; H, 5.81; N, 7.31. $C_{56}H_{44}N_4$ requires C, 87.01; H, 5.74; N, 7.25%; mp 176 °C; IR (KBr, cm^{-1}) 1698, 1642, 1600; δ_H (400 MHz, $CDCl_3$) 4.86 (s, 8H, CH_2N), 7.39 (d, 8H, J=8.2 Hz), 7.43–7.51 (m, 4H), 7.56 (d, 2H, J=1.6 Hz), 7.57 (t, 2H, J=1.4 Hz), 7.60 (d, 10H, J=8.2 Hz), 7.75 (t, 2H, J=1.4 Hz), 7.84 (2H, J=1.6 Hz), 7.85 (2H, J=1.8 Hz), 8.45 (s, 4H, CHN); δ_C (100 MHz, $CDCl_3$) 64.9, 126.2, 127.6, 128.7, 129.1, 129.4, 130.0, 130.5, 136.7, 138.5, 140.1, 141.7, 161.6; MS (ES) m/z: 773.4 $[M+H]^+$.

4.3. General procedure for the preparation of tetraamines

Sodium borohydride (40 mmol) was added to a solution of tetraimine (6.5 mmol) in mixture of toluene (375 mL), THF (375 mL), and methanol (375 mL) at 0–5 °C. The reaction mixture was stirred at 0–5 °C for 4 h. To the reaction mixture cold water (750 mL) was added followed by DCM (750 mL). The layers were separated and aqueous layer was extracted with DCM (2×250 mL). The combined organic layers were washed with brine solution and then dried over anhydrous potassium carbonate. Removal of the DCM under reduced pressure gave the corresponding cyclophane tetraamine as a crude material, which was purified by column chromatography (SiO_2).

*4.3.1. Tetraamine **15**.* The crude product was purified by column chromatography (1% MeOH/$CHCl_3$) to give the tetraamine **15** as off-white solid (2.45 g, 80%), Found: C, 80.86; H, 7.68; N, 11.81. $C_{32}H_{36}N_4$ requires C, 80.63; H, 7.61; N, 11.75%; R_f (1% MeOH/$CHCl_3$) 0.40; mp 147 °C; IR (KBr, cm^{-1}) 1442; δ_H (400 MHz, $CDCl_3$) 1.65 (br s, 4H, NH), 3.78 (s, 16H, CH_2N), 7.20 (t, 2H, J=1.6 Hz), 7.22 (s, 6H), 7.26–7.29 (m, 8H); δ_C (100 MHz, $CDCl_3$) 53.8, 127.2, 128.0, 128.6, 140.6; MS (ES) m/z: 477.2 $[M+H]^+$.

*4.3.2. Tetraamine **18**.* The crude product was purified by column chromatography (1% MeOH/$CHCl_3$) to give the tetraamine **18** as off-white solid (2.45 g, 80%). Found: C, 86.60; H, 6.82; N, 7.28. $C_{56}H_{52}N_4$

Table 5
Anti-bacterial activity of cyclophane amides **1**–**13** by cup plate method

Cyclophane amide	Concentration in μg/mL	Diameter of zone of inhibition in mm			
		E. coli	*Staphylococcus aureus*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*
1	250	—	—	—	—
	500	—	—	—	—
	1000	11.00±1.00	—	—	—
	2000	12.03±1.04	—	—	—
2	250	—	—	—	—
	500	—	—	—	—
	1000	11.00±0.57	—	—	—
	2000	12.10±1.00	—	—	—
3	250	—	—	11.00±0.63	—
	500	—	—	12.00±0.55	—
	1000	—	—	12.00±1.05	—
	2000	—	—	13.30±0.57	—
4	250	—	12.00±1.00	—	—
	500	—	12.00±1.15	—	—
	1000	—	13.00±1.00	—	—
	2000	—	14.00±1.01	—	—
5	250	—	11.00±1.02	—	—
	500	—	12.00±0.58	—	—
	1000	—	14.00±0.56	—	—
	2000	—	15.00±0.58	—	—
6	250	—	—	14.10±1.02	—
	500	—	—	15.00±0.55	—
	1000	—	—	—	—
	2000	—	—	—	—
7	250	—	—	10.00±1.03	—
	500	—	—	12.00±1.02	—
	1000	—	—	15.00±1.00	—
	2000	—	—	15.33±0.56	—
Standard	250	20.00±0.58	27.00±1.00	18.00±0.56	16.00±1.00

Each value represents mean±SD of three observations.

requires C, 86.12; H, 6.71; N, 7.17%; R_f (1% MeOH/$CHCl_3$) 0.55; mp 168 °C; IR (KBr, cm^{-1}) 1642; δ_H (400 MHz, $CDCl_3$) 1.72 (br s, 4H, N*H*), 3.85 (s, 16H, CH_2N), 7.27 (merged with $CDCl_3$, 3H), 7.30–7.35 (m, 5H), 7.39 (d, 8H, *J*=7.9 Hz), 7.43 (d, 2H, *J*=6.8 Hz), 7.49 (d, 4H, *J*=7.5 Hz), 7.54 (d, 8H, *J*=7.9 Hz), 7.71 (s, 2H); δ_C (100 MHz, DMSO-d_6) 51.7, 52.1, 124.8, 125.5, 126.5, 126.6, 127.6, 128.0, 128.6, 128.8, 129.4, 138.5, 139.8, 140.2, 140.7; MS (ES) *m*/*z*: 781.5 $[M+H]^+$.

4.4. General procedure for the synthesis of cyclophane amides

A solution of diamine (7.3 mmol) in dry dichloromethane (400 mL) and a solution of the corresponding diacid chloride (7.3 mmol)/disulfonyl chloride (7.3 mmol) in dichloromethane (400 mL) were simultaneously added dropwise to a well stirred solution of triethylamine (23.7 mmol) in dry dichloromethane (1000 mL) for 8 h. After the addition was complete, the reaction mixture was stirred for another 24 h. The solvent was removed at reduced pressure and the solid obtained was washed with water (2×200 mL) to remove triethylammonium chloride, which was purified by column chromatography (SiO_2).

*4.4.1. Cyclophane amide **1**.* The crude product was purified by column chromatography (5% MeOH/$CHCl_3$) to give the cyclophane amide **1** as off-white solid (1.56 g, 80%). Found: C, 72.46; H, 5.38; N, 10.61. $C_{32}H_{28}N_4O_4$ requires C, 72.16; H, 5.30; N, 10.52%; R_f (5% MeOH/$CHCl_3$) 0.40; mp >370 °C; IR (KBr, cm^{-1}) 1638, 1542, 1303; δ_H (400 MHz, DMSO-d_6) 4.44 (d, 8H, *J*=5.9 Hz, CH_2N), 7.19–7.30 (m, 8H), 7.48 (t, 2H, *J*=7.7 Hz), 7.95–7.99 (m, 4H), 8.28 (s, 2H), 9.08 (t, 4H, *J*=5.8 Hz, N*H*); δ_C (100 MHz, DMSO-d_6) 42.7, 125.2, 126.1, 126.6, 128.0, 128.3, 129.8, 134.1, 134.5, 139.6, 139.8, 165.3, 165.8; MS (ES) *m*/*z*: 533 $[M+H]^+$.

*4.4.2. Cyclophane amide **2**.* The crude product was purified by column chromatography (5% MeOH/$CHCl_3$) to give the cyclophane amide **2** as off-white solid (1.56 g, 80%). Found: C, 67.56; H, 4.98; N, 15.93. $C_{30}H_{26}N_6O_4$ requires C, 67.40; H, 4.90; N, 15.72%; R_f (5% MeOH/$CHCl_3$) 0.35; mp 259 °C; IR (KBr, cm^{-1}) 1658, 1534, 1445; δ_H (400 MHz, DMSO-d_6) 4.48 (br s, 8H, CH_2N), 7.11–7.21 (m, 8H), 8.06–8.18 (m, 6H), 9.80 (br s, 4H, N*H*); δ_C (100 MHz, DMSO-d_6) 42.1, 124.3, 125.5, 128.4, 139.3, 139.5, 148.5, 163.3; MS (FAB^+) *m*/*z*: 534 $[M]^+$.

*4.4.3. Cyclophane sulfonamide **3**.* The crude product was purified by column chromatography (5% MeOH/$CHCl_3$) to give the cyclophane amide **3** as off-white solid (1.49 g, 60%). Found: C, 49.91; H, 4.23; N, 8.35. $C_{28}H_{28}N_4O_8S_4$ requires C, 49.69; H, 4.17; N, 8.28%; R_f (5% MeOH/$CHCl_3$) 0.40; mp 250 °C (decomposed); IR (KBr, cm^{-1}) 1631, 1455, 1424; δ_H (400 MHz, DMSO-d_6) 4.21 (d, 8H, *J*=6.2 Hz, CH_2N), 6.83 (d, 4H, *J*=7.4 Hz), 6.91 (t, 2H, *J*=7.6 Hz), 7.14 (s, 2H), 7.34 (t, 2H, *J*=7.8 Hz), 7.53 (d, 4H, *J*=7.7 Hz), 8.07 (s, 2H), 8.37 (t, 4H, *J*=6.2 Hz, N*H*); δ_C (100 MHz, DMSO-d_6) 47.1, 127.5, 129.1, 129.4, 129.8, 130.9, 133.6, 141.8; MS (ES) *m*/*z*: 677.6 $[M+H]^+$.

*4.4.4. Cyclophane amide **8**.* The crude product was purified by column chromatography (5% MeOH/$CHCl_3$) to give the cyclophane amide **8** as off-white solid (1.22 g, 80%). Found: C, 80.56; H, 5.36; N, 6.75. $C_{54}H_{42}N_6O_4$ requires C, 80.36; H, 5.30; N, 6.69%; R_f (5% MeOH/$CHCl_3$) 0.45; mp 185 °C; IR (KBr, cm^{-1}) 1641, 1534, 1477; δ_H (400 MHz, DMSO-d_6) 4.54 (br s, 8H, CH_2N), 7.43 (d, 8H, *J*=6.3 Hz), 7.53 (t, 2H, *J*=7.5 Hz), 7.60–7.63 (m, 6H), 7.67–7.77 (m, 7H), 7.79 (s, 1H), 7.85 (br s,

1H), 8.05–8.11 (m, 4H), 8.31 (d, 1H, J=2.1 Hz), 8.45 (s, 1H), 8.49 (s, 1H), 9.19–9.24 (m, 4H, N*H*); δ_C (100 MHz, DMSO-d_6) 42.5, 124.9, 125.6, 126.8, 126.9, 128.0, 128.1, 129.5, 129.9, 130.0, 131.9, 134.4, 134.6, 138.7, 139.0, 139.1, 140.7, 165.6, 165.9; MS (ES) m/z: 835.6 [M−1].

4.4.5. Cyclophane amide ***9***. The crude product was purified by column chromatography (5% MeOH/$CHCl_3$) to give the cyclophane amide **9** as off-white solid (1.22 g, 80%). Found: C, 77.56; H, 5.15; N, 10.07. $C_{56}H_{44}N_4O_4$ requires C, 77.31; H, 5.05; N, 10.02%; R_f (5% MeOH/$CHCl_3$) 0.40; mp 198 °C; IR (KBr, cm^{-1}) 1663, 1530, 1444; δ_H (400 MHz, DMSO-d_6) 4.65 (br s, 8H, CH_2N), 7.30–7.48 (m, 9H), 7.52–7.61 (m, 4H), 7.68–7.75 (m, 8H), 7.78–7.86 (m, 2H), 8.21–8.32 (m, 7H), 9.97 (br s, 4H, N*H*); δ_C (100 MHz, DMSO-d_6) 42.1, 124.6, 124.9, 125.0, 125.5, 126.9, 127.7, 128.1, 128.2, 129.5, 138.8, 139.6, 139.9, 140.6, 148.7, 149.3, 163.4; MS (ES) m/z: 838 $[M]^+$.

4.4.6. Cyclophane amide ***10***. The crude product was purified by column chromatography (5% MeOH/$CHCl_3$) to give the cyclophane amide **10** as off-white solid (1.20 g, 70%). Found: C, 82.76; H, 5.35; N, 5.71. $C_{34}H_{26}N_2O_2$ requires C, 82.57; H, 5.30; N, 5.66%; R_f (5% MeOH/$CHCl_3$) 0.35; mp 260 °C (decomposed); IR (KBr, cm^{-1}) 1639, 1607, 1487; δ_H (400 MHz, DMSO-d_6) 4.56 (s, 4H, CH_2N), 7.44 (t, 4H, J=8.3 Hz), 7.72 (d, 4H, J=7.9 Hz), 7.88 (d, 6H, J=8.2 Hz), 8.04–8.27 (m, 6H), 9.20 (t, 2H, J=5.5 Hz, N*H*); MS (ES) m/z: 495.1 $[M+H]^+$.

4.4.7. Cyclophane sulfonamide ***11***. The crude product was purified by column chromatography (5% MeOH/$CHCl_3$) to give the cyclophane amide **11** as off-white solid (1.19 g, 70%). Found: C, 63.86; H, 4.57; N, 5.79%. $C_{52}H_{44}N_4O_8S_4$ requires C, 63.65; H, 4.52; N, 5.71%; R_f (5% MeOH/$CHCl_3$) 0.40; mp 236 °C; IR (KBr, cm^{-1}) 1603, 1517, 1478; δ_H (400 MHz, DMSO-d_6) 4.07 (d, 8H, J=5.8 Hz, CH_2N), 7.13 (d, 2H, J=8.2 Hz), 7.29 (d, 4H, J=7.8 Hz), 7.46 (d, 3H, J=8.1 Hz), 7.49–7.57 (m, 6H), 7.61 (d, 5H, J=7.1 Hz), 7.70 (d, 1H, J=7.8 Hz), 7.74 (d, 1H, J=7.9 Hz), 7.77 (s, 2H), 7.81–7.86 (m, 2H), 7.93 (dd, 1H, J=1.7 Hz), 7.99 (d, 2H, J=7.7 Hz), 8.12 (s, 1H), 8.24 (s, 1H), 8.29 (t, 1H, J=6.3 Hz), 8.51 (ABq, 4H, J=5.8 Hz, N*H*); δ_C (100 MHz, DMSO-d_6) 47.1, 127.5, 129.1, 129.4, 129.8, 130.9, 133.6, 141.8; MS (ES) m/z: 981.2 $[M+H]^+$.

4.5. General procedure for the synthesis of tricyclic cyclophane amides

A solution of tetraamine (1.05 mmol) in dry dichloromethane (250 mL) and a solution of the corresponding diacid chloride (2.10 mmol) in dichloromethane (250 mL) were simultaneously added dropwise to a well stirred solution of triethylamine (4.2 mmol) in dry dichloromethane (500 mL) for 6 h. After the addition was complete, the reaction mixture was stirred for another 24 h. The solvent was removed at reduced pressure and the residue obtained was then dissolved in chloroform (250 mL), washed with water (2×200 mL) to remove triethylammonium chloride, and then dried over anhydrous sodium sulfate. Removal of the chloroform under reduced pressure gave the corresponding cyclophane amide as a crude material, which was purified by column chromatography (SiO_2).

4.5.1. Cyclophane amide ***4***. The crude product was purified by column chromatography (2% MeOH/$CHCl_3$) to give the cyclophane amide **4** as a white solid (0.50 g, 65%). Found: C, 78.43; H, 5.51; N, 7.72. $C_{48}H_{40}N_4O_4$ requires C, 78.24; H, 5.47; N, 7.60%; R_f (2% MeOH/$CHCl_3$) 0.45; mp 176 °C; IR (KBr, cm^{-1}) 1639, 406; δ_H (400 MHz, $CDCl_3$) 4.10 (d, 8H, J=14.7 Hz, CH_2N), 4.81 (d, 8H, J=14.7 Hz, CH_2N), 7.15 (d, 8H, J=7.5 Hz), 7.23 (d, 4H, J=7.5 Hz), 7.63 (ABq, 12H, J=7.6 Hz); δ_C (100 MHz, $CDCl_3$) 45.2, 49.4, 51.0, 53.6, 57.1, 124.8, 125.5, 127.1, 127.1, 127.2, 127.8, 128.0, 128.4, 128.9, 129.0, 129.2, 130.0, 130.7, 131.2, 136.3, 137.4, 137.5, 140.3, 160.7, 170.1, 172.7, 174.3; MS (ES) m/z: 737.2 $[M+H]^+$.

4.5.2. Cyclophane amide ***5***. The crude product was purified by column chromatography (2% MeOH/$CHCl_3$) to give the cyclophane amide **5** as off-white solid (0.54 g, 70%). Found: C, 74.92; H, 5.31; N, 11.47. $C_{46}H_{38}N_6O_4$ requires C, 74.78; H, 5.18; N, 11.37%; R_f (2% MeOH/$CHCl_3$) 0.40; mp 370 °C (decomposed); IR (KBr, cm^{-1}) 1631, 1421; δ_H (400 MHz, $CDCl_3$) 3.80 (d, 4H, J=14.2 Hz, CH_2N), 5.00 (d, 4H, J=15.7 Hz, CH_2N), 5.10 (d, 4H, J=15.5 Hz, CH_2N), 5.41 (d, 4H, J=14.1 Hz, CH_2N), 6.96 (t, 6H, J=7.5 Hz), 7.06 (d, 4H, J=6.8 Hz), 7.23–7.25 (m, 2H), 7.37 (s, 2H), 7.94 (t, 8H, J=12.8 Hz); δ_C (100 MHz, $CDCl_3$) 50.8, 55.0, 125.7, 126.2, 127.0, 127.5, 128.1, 128.5, 129.1, 137.1, 138.3, 138.6, 152.4, 169.2; MS (ES) m/z: 739.3 $[M+H]^+$.

4.5.3. Cyclophane amide ***6***. The crude product was purified by column chromatography (2% MeOH/$CHCl_3$) to give the cyclophane amide **6** as a white solid (0.47 g, 50%). Found: C, 81.32; H, 5.51; N, 6.38%. $C_{60}H_{48}N_4O_4$ requires C, 81.06; H, 5.44; N, 6.30%; R_f (2% MeOH/$CHCl_3$) 0.35; mp 260 °C; IR (KBr, cm^{-1}) 1634, 1406; δ_H (400 MHz, $CDCl_3$) 3.20 (br s, 2H, CH_2N), 3.48 (distorted doublet, 6H, CH_2N), 4.50 (br s, 2H, CH_2N), 5.34 (distorted doublet, 6H, CH_2N), 6.35 (t, 4H, J=7.2 Hz), 6.59 (s, 4H), 6.73 (s, 2H), 7.14 (d, 8H, J=9.8 Hz), 7.33 (t, 4H, J=7.5 Hz), 7.45–7.61 (m, 8H), 7.74 (br s, 2H); δ_C (100 MHz, $CDCl_3$) 45.5, 52.1, 52.6, 55.1, 124.3, 125.8, 126.2, 127.1, 128.1, 128.5, 128.8, 129.1, 129.7, 131.2, 132.9, 134.8, 135.3, 137.0, 138.4, 143.0, 170.3, 171.3; MS (ES) m/z: 889.3 $[M+H]^+$.

4.5.4. Cyclophane sulfonamide ***7***. The crude product was purified by column chromatography (2% MeOH/$CHCl_3$) to give the cyclophane amide **7** as off-white solid (0.69 g, 75%). Found: C, 60.23; H, 4.65; N, 6.42. $C_{44}H_{40}N_4O_8S_4$ requires C, 59.98; H, 4.58; N, 6.36%; R_f (2% MeOH/$CHCl_3$) 0.30; mp 370 °C (decomposed); IR (KBr, cm^{-1}) 1624, 1449, 1334; δ_H NMR (400 MHz, DMSO-d_6) 3.65 (s, 4H, CH_2N), 4.17 (s, 4H, CH_2N), 4.37 (s, 8H, CH_2N), 7.15–7.18 (m, 5H), 7.24–7.29 (m, 8H), 7.53 (d, 1H, J=7.8 Hz), 7.60 (d, 2H, J=7.5 Hz), 7.66 (t, 2H, J=7.7 Hz), 7.83 (s, 1H), 7.94 (t, 3H, J=8.8 Hz), 9.13 (br s, 2H); δ_C (100 MHz, DMSO-d_6) 45.6, 48.9, 49.3, 51.8, 124.0, 127.3, 128.3, 128.7, 128.9, 129.1, 129.2, 129.3, 129.5, 129.6, 130.1, 130.4, 130.8, 131.3, 132.0, 132.3, 136.8, 138.5, 149.4; MS (ES) m/z: 881.1 $[M+H]^+$.

4.5.5. Cyclophane amide ***12***. The crude product was purified by column chromatography (2% MeOH/$CHCl_3$) to give the cyclophane amide **12** as a white solid (0.56 g, 45%). Found: C, 84.81; H, 5.50; N, 4.76. $C_{84}H_{64}N_4O_4$ requires C, 84.54; H, 5.41; N, 4.69%; R_f (2% MeOH/$CHCl_3$) 0.40; mp 272 °C; IR (KBr, cm^{-1}) 1634, 1597; δ_H (400 MHz, $CDCl_3$) 3.80, 3.84 (a pair of doublets, 4H, J=1.7 Hz, CH_2N), 4.62 (q, 8H, J=14.8, 16.1 Hz, CH_2N), 4.83, 4.87 (a pair of doublets, 4H, J=8.1, 7.9 Hz, CH_2N), 6.71 (s, 1H), 6.76 (s, 1H), 6.79–6.86 (m, 4H), 6.96 (t, 1H, J=7.6 Hz), 7.02 (t, 1H, J=7.5 Hz), 7.09, 7.11 (two doublets, 8H, J=2.2 Hz), 7.14–7.18 (m, 8H), 7.32 (d, 2H, J=1.3 Hz), 7.39 (d, 6H, J=0.9 Hz), 7.42–7.47 (m, 5H), 7.50–7.55 (m, 7H), 8.31 (d, 4H, J=7.9 Hz); δ_C (100 MHz, $CDCl_3$) 49.0, 52.5, 125.8, 125.9, 126.1, 127.0, 127.7, 127.9, 128.2, 129.2, 129.3, 129.4, 134.7, 134.8, 137.1, 137.5, 137.6, 138.2, 140.0, 141.6, 170.7; MS (ES) m/z: 1193.7 $[M+H]^+$.

4.5.6. Cyclophane amide ***13***. The crude product was purified by column chromatography (2% MeOH/$CHCl_3$) to give the cyclophane amide **13** as a white solid (0.63 g, 50%). Found: C, 84.82; H, 5.48; N, 4.76. $C_{84}H_{64}N_4O_4$ requires C, 84.54; H, 5.41; N, 4.69%; R_f (2% MeOH/$CHCl_3$) 0.35; mp 282 °C; IR (KBr, cm^{-1}) 1634, 1597; δ_H (400 MHz, $CDCl_3$) 3.80, 3.84 (two d, 4H, J=1.7 Hz, CH_2N), 4.62 (ABq, 8H, J=14.8, 16.1 Hz, CH_2N), 4.85 (q, 4H, J=8.0 Hz, CH_2N), 6.71 (s, 1H), 6.76 (s, 1H), 6.79–6.86 (m, 4H) 6.96 (t, 1H, J=7.6 Hz), 7.02 (t, 1H, J=7.5 Hz), 7.09, 7.11 (two d, 8H, J=2.2 Hz), 7.14–7.18 (m, 8H), 7.32 (d, 2H, J=1.3 Hz), 7.39 (d, 6H, J=0.9 Hz), 7.42–7.47 (m, 5H), 7.50–7.55 (m, 7H), 8.31 (d,

4H, J=7.9 Hz); δ_C (100 MHz, $CDCl_3$) 49.0, 52.5, 125.8, 125.9, 126.1, 127.0, 127.7, 127.9, 128.2, 129.2, 129.3, 129.4, 134.7, 134.8, 137.1, 137.5, 137.6, 138.2, 140.0, 141.6, 170.7; MS (ES) m/z: 1193.7 $[M+H]^+$.

4.6. Biological activity

4.6.1. In vitro anti-inflammatory assay. The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity using prednisolone as standard drug. Blood was collected from healthy volunteers and the collected blood was mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride). The blood was centrifuged at 1500 rpm and the packed cells were washed with isotonic sodium chloride (0.85%, pH 7.2) and a 10% v/v suspension of the packed cells was made with isotonic sodium chloride. The assay mixture contains the cyclophane amides dissolved in DMSO (200, 400, and 800 μg/mL), Phosphate buffer (1 mL, 0.15 M, pH 7.4), hypotonic sodium chloride (2 mL, 0.36%), and HRBC suspension (0.5 mL). Prednisolone (10, 50, 100, and 200 μg/mL) was used as the reference drug. Instead of hypotonic sodium chloride, distilled water (2 mL) was used in the control. All the assay mixtures were incubated at 37 °C for 30 min. and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer (Systronic UV–vis Spectrophotometer 118) at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the formula.

$$\%\text{Protection} = 100 - \{[(\text{Optical Density of test solution} - \text{Optical Density of product control}) \div (\text{Optical Density of test control})]\} \times 100$$

The lysosomal enzyme released during inflammation produced a variety of disorders. This extracellular activity of this enzyme is related to acute or chronic inflammation. Since the HRBC membrane components are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis taken as a measure of anti-inflammatory activity of the drug.

4.6.2. In vitro anti-bacterial assay. The anti-bacterial studies were carried out aseptically under in vitro conditions by 'cup plate method'. The authentic bacterial strains were inoculated in nutrient broth overnight and used. The sterile nutrient agar media at 40–50 °C was transferred aseptically to sterile Petri plates and allowed to solidify. The bacterial cultures were then inoculated by swabbing technique. Borers of 8 mm diameter were made on the bacteria seeded agar. The various fractions of the drug are dissolved in DMSO, so as to contain 250, 500, 1000, 2000 μg/mL of the drug. Each drug solution (100 μL) was added to the respective cups along with standard Gentamycin (5 μg), Ciprofloxacin (5 μg), Cefotaxime (10 μg) for *E. coli*, *Staphylococcus*, *Klebsiella*, *Pseudomonas*, respectively, along with the solvent control DMSO. The bacteria seeded agar plates were aseptically transferred to incubator and incubated at 37 °C for 18–24 h. The diameter of the zone of inhibition was measured after 12 and 24 h of inhibition and compared with standard antibiotics.

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Supplementary data

X-ray crystal data for cyclophane amide 5 (Table 1) is available. Crystallographic data for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 743880 and CCDC 743881. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [e-mail: deposit@ccdc.cam.ac.uk]. Supplementary data associated with this article can be found in online version, at doi:10.1016/j.tet.2011.10.046.

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