CSIRO PUBLISHING

Aust. J. Chem. **2012**, *65*, 186–192
http://dx.doi.org/10.1071/CH11435

Full Paper

Synthesis, Anti-Arthritic, and Anti-Inflammatory Activity of *N*-Tosyl aza Cyclophanes

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The synthesis of novel *N*-tosyl tetraaza cyclophanes and *N*-tosyl diaza cyclophane incorporating *m*-terphenyl as spacer units is described. Anti-arthritic activity was studied by inhibition of the protein denaturation method (bovine serum albumin). All the *N*-tosyl aza cyclophanes exhibit excellent anti-arthritic activity. Anti-inflammatory activity of the synthesized cyclophanes was investigated using the human red blood cells (HRBC) membrane stabilization method and some of the *N*-tosyl aza cyclophanes exhibited good anti-inflammatory activity.

Manuscript received: 13 November 2011.
Manuscript accepted: 9 January 2012.
Published online: 1 February 2012.

Introduction

Transfer of information between molecules in living systems and in supramolecular structures takes place mainly through non-covalent interactions. Molecular recognition in biology plays an important role in the development of supramolecular chemistry. The aza-crown macrocycles are used as synthetic receptors in anion complexation processes that are similar to those in biological systems.[1] Furthermore, in supramolecular chemistry the polyaza macrocycles are highly interesting systems as they can be used for the binding of both organic and inorganic ions.[2,3] The intra annular functionality present in the macrocycles enhances the molecular recognition, metal binding properties and biological activity.[4] Introducing functional groups such as amides and esters in the aza-crown macrocyclic system would make them models of protein-metal binding sites in biological systems.[5–7] Sulfone derivatives[8] are well known for their biological activities such as antimicrobial,[9] anti-inflammatory,[10] and inhibition of HIV-1 reverse transcription.[11] Synthesis and characterization of macrocyclic aromatic tetrasulfonates and polyaza macrocycles were recently reported.[12]

Sulfonamides incorporated with acylic receptors and their complexation with anionic guests by hydrogen bonding have also been reported.[13–15] Supramolecular triazadisulfonamides and their anti-HIV studies have been also recently reported by Pinheiro et al.[16] Sulfonamides are the basis of several groups of drugs. The sulfonamide chemical moiety is present in antibacterials (sulfa drugs), thiazide diuretics (including hydrochlorothiazide, metolazone, and indapamide), loop diuretics (including furosemide, bumetanide and torsemide) sulfonylureas (including glipizide, glyburide), some COX-2 inhibitors (e.g. celecoxib, etriocoxib, rofecoxib, parecoxib and valdecoxib) and acetazolamide.

Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by counteracting the cyclooxygenase (COX) enzyme that converts arachidonic acid into prostaglandins in inflammatory processes.[17] NSAIDs are used for the treatment of pain, fever, and inflammation, particularly arthritis.[18] The common NSAIDs such as aspirin, ibuprofen, naproxen and fenbufen have side effects such as upper gastrointestinal (GI) irritation, ulceration, dyspepsia, bleeding, in some cases death and give only temporary relief.[19] To overcome the GI ulceration side effect of these drugs, more COX-2-selective inhibitor NSAIDs are preferred, which do not significantly inhibit cyclooxygenase in the stomach and appear to be less likely to cause GI ulceration.[20] Unfortunately very effective COX-2 selective inhibitor drugs, i.e. rofecoxib and celecoxib, were withdrawn from the market because of the increased risk of heart attack and stroke associated with long-term, high-dose use. The COX-2 inhibition activity of terphenyl analogues was reported by Li et al.[21] Hence considerable attention has been focussed to synthesize and study the anti-arthritic and anti-inflammatory activity of novel cyclophane sulfonamides.

We have recently reported the synthesis of cyclophane amides with anti-inflammatory and anti-bacterial efficacy,[22] and carbazole based macrocyclic amides with antimicrobial activity.[23] In continuation of our ongoing investigation on the synthesis of various bioactive cyclophanes, attempts were made to synthesize novel *N*-tosyl tetraaza cyclophanes and *N*-tosyl diaza cyclophanes by coupling between suitable dibromides and bis(tosylaminomethyl)*m*-terphenyl, and then to study the bioactivity of the synthesized cyclophanes. However, to the best of

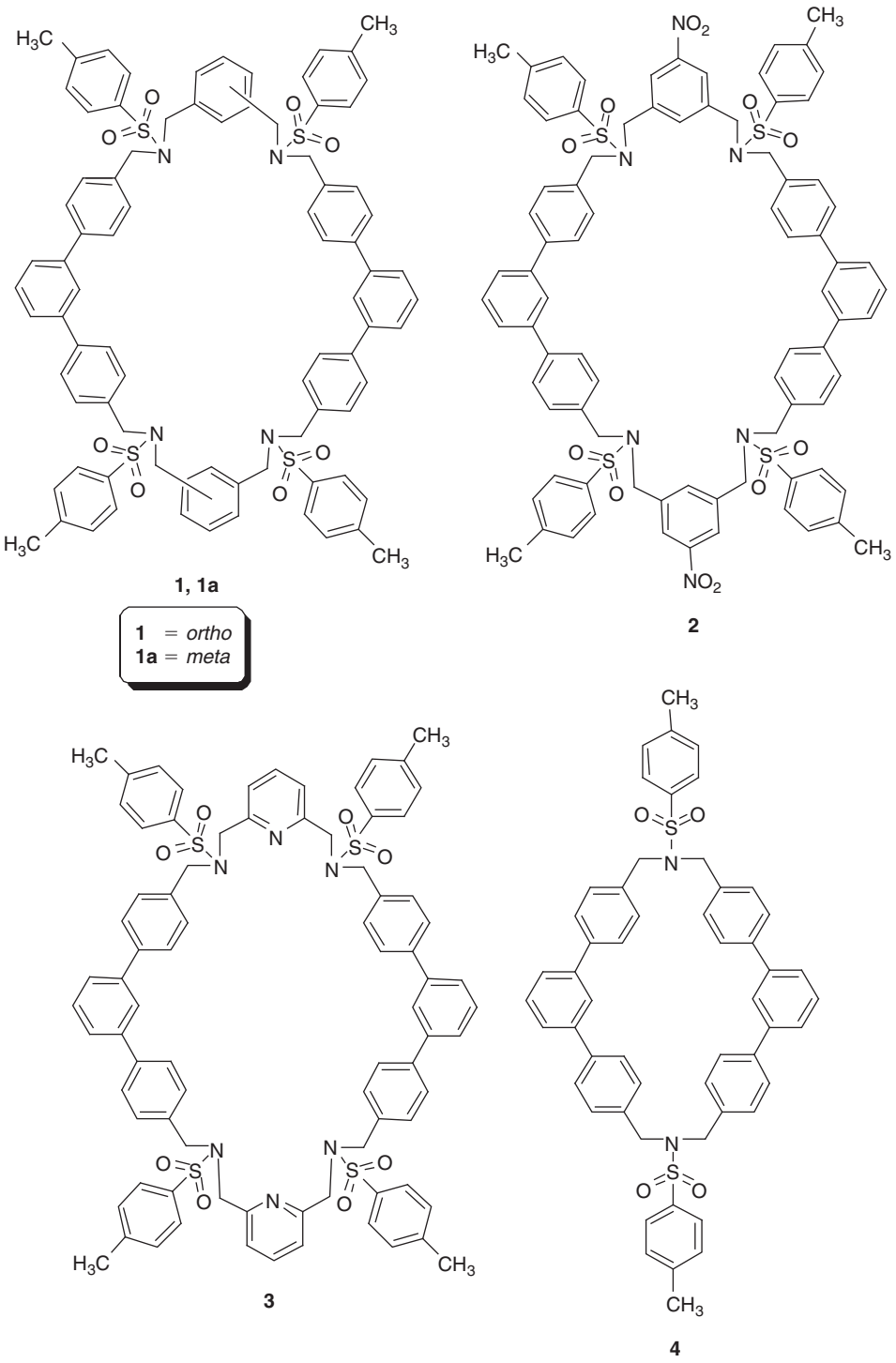
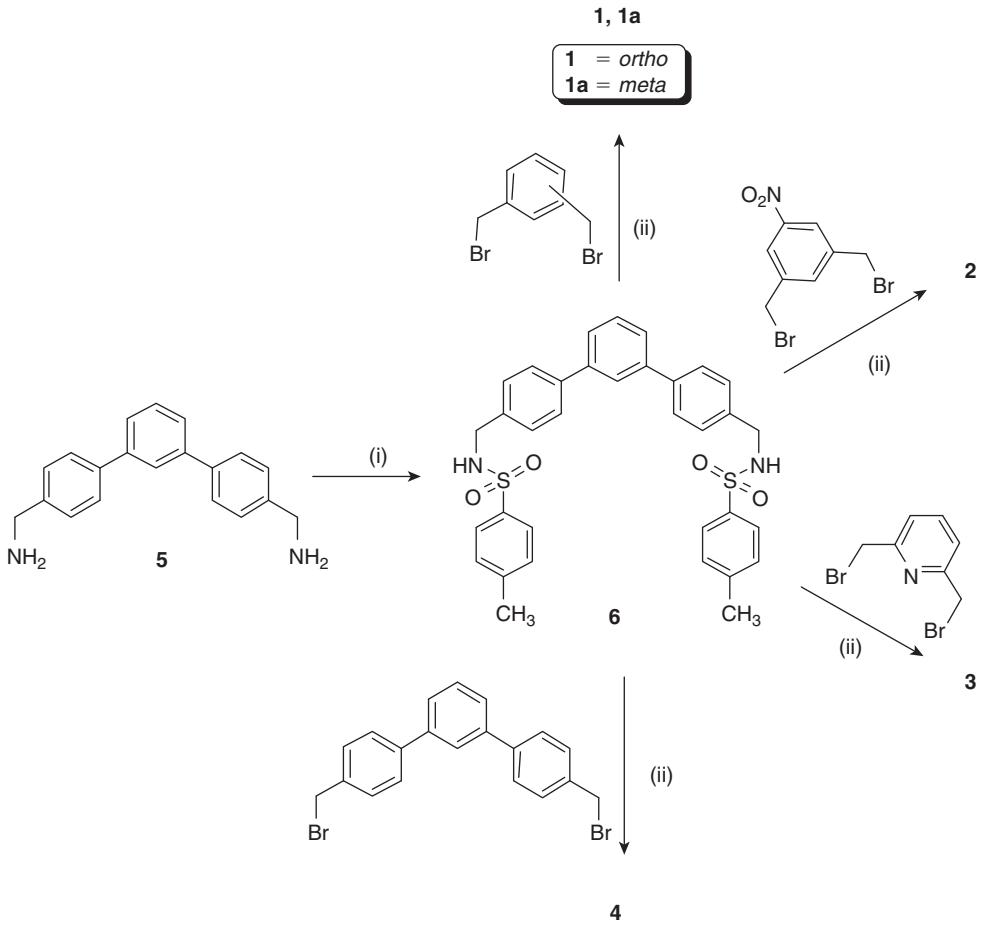


Fig. 1. Structure of *N*-tosyl tetraaza cyclophanes **1**–**3** and *N*-tosyl diaza cyclophane **4**.

our knowledge, such cyclophanes with bis(tosylaminomethyl)*m*-terphenyl as the spacer have not been reported in the literature. Herein, we report the synthesis, anti-arthritic and anti-inflammatory activity of *N*-tosyl tetraaza cyclophanes **1**–**3** and *N*-tosyl diaza cyclophane **4** (Fig. 1).

Results and Discussion

N-tosyl tetraaza cyclophanes (**1**–**3**) and *N*-tosyl diaza cyclophane (**4**) (Fig. 1) were synthesized with bis(tosylaminomethyl) *m*-terphenyl **6**[24] and possible combinations of *o*-xylylene dibromide, *m*-xylylene dibromide, *m*-nitro xylylene dibromide, 2,6-(bisbromomethyl)pyridine and *m*-terphenyl dibromide as building units. Bis(tosylaminomethyl)*m*-terphenyl **6** required for the synthesis of *N*-tosyl tetraaza cyclophanes **1**–**3** and *N*-tosyl diaza cyclophane, **4** was obtained in 85 % yield by the reaction of 1 equiv *m*-terphenyl diamine **5** with 2.1 equiv *p*-toluene sulfonyl chloride in the presence of 2.2 equiv pyridine in dry chloroform for 5 h at 0–5°C (Scheme 1). The ^{1}H NMR spectrum of precyclophane **6** displayed a singlet for the methyl protons at



Scheme 1. Reagents and conditions: (i) *p*-toluene sulfonyl chloride, pyridine, $CHCl_3$, 0–5°C, 5 h, **6** (85 %); (ii) K_2CO_3, ACN, rt, 48 h, **1** (68 %), **1a** (68 %), **2** (50 %), **3** (40 %) and **4** (80 %).

δ 2.44, a doublet for the *N*-methylene protons at δ 4.19 and a triplet for NH protons at δ 4.63. The remaining aromatic protons resonated between δ 7.18 and 7.80. The structure of precyclophane **6** was further confirmed from the spectral and analytical data.

One equiv of precyclophane **6** was coupled with 1 equiv of *o*-xylylene dibromide, *m*-xylylene dibromide, *m*-nitro xylylene dibromide, 2,6-(bisbromomethyl)pyridine or *m*-terphenyl dibromide in the presence of anhydrous potassium carbonate in dry acetonitrile at room temperature under high dilution conditions. The reaction afforded the *N*-tosyl tetraaza cyclophanes **1**, **1a**, **2**, **3** and *N*-tosyl diaza cyclophane **4** in 68, 68, 50, 40 and 80 % yields respectively, after purification by column chromatography (Scheme 1). The structures of the *N*-tosyl tetraaza and diaza cyclophanes **1**–**4** were confirmed using spectral and analytical data. The ^{1}H NMR spectrum of *N*-tosyl tetraaza cyclophane **1a** displayed a singlet for the methyl protons at δ 2.45 and a singlet for the *N*-methylene protons at δ 4.17. The rest of the aromatic protons resonated between δ 6.67 and 7.77. In the ^{13}C NMR spectrum of *N*-tosyl tetraaza cyclophane **1a**, the *N*-methylene carbons appeared at δ 50.7 and 51.1. The FT-IR spectrum of **1a** displayed the sulfonyl stretching frequency at 1597 cm^{-1} and the mass spectrum showed the molecular ion peak $[(M+NH_4)^+]$ at *m/z* 1414.6. Similarly the structure of the *N*-tosyl tetraaza and diaza cyclophanes **1**, **2**, **3** and **4** was also confirmed from spectral and analytical data.

In vitro anti-arthritic activity was studied by inhibition of the protein denaturation method (bovine serum albumin [BSA]).[25] The denaturation of protein is one of the causes of rheumatoid arthritis.[26] Production of auto-antigens in certain rheumatic diseases may be owing to in vivo denaturation of proteins.[27] The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulfide bonding.[28] The observed data showing the anti-arthritic activity of the compounds and the control drug are given in Table 1 and Fig. 2. In order to study the anti-arthritic activity of the *N*-tosyl tetraaza cyclophanes **1**, **1a**, **2**, **3** and *N*-tosyl diaza cyclophane **4** inhibition of the protein denaturation method was employed (BSA) and diclofenac sodium was used as a standard. The results are shown in Table 1. The anti-arthritic activities of all of the *N*-tosyl tetraaza and diaza macrocycles are concentration dependent. At the higher concentration, the *N*-tosyl tetraaza and diaza macrocycles **1a**, **2**, **3** and **4** exhibited better anti-arthritic activity than at lower concentration. All the compounds **1**, **1a**, **2**, **3** and **4** were found to possess the maximum anti-arthritic activity (74.42, 82.02, 93.75, 86.85 and 78.63 % at 800 $\mu g\,mL^{-1}$) when compared with the reference drug diclofenac sodium (76.46 % at 800 $\mu g\,mL^{-1}$), which clearly shows that *N*-tosyl tetraaza and diaza macrocycles are superior to the reference drug diclofenac sodium. The superior inhibition of protein denaturation results showed that the *N*-tosyl tetraaza and diaza macrocycles are more stable than dicofenac sodium in BSA. It is possible that the stability related to the strong binding of the

Table 1. In vitro anti-arthritic activity of *N*-tosyl tetraaza and diaza cyclophanes 1–4 by inhibition of protein denaturation method (bovine serum albumin)

Each value represents mean ± s.d. of three observations

Cyclophane sulfonamide	Activity (% inhibition of protein denaturation)				
	50 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}	400 μg mL^{-1}	800 μg mL^{-1}
1	13.60	24.59	47.09	62.12	74.42
1a	23.68	40.95	57.90	78.17	82.02
2	22.63	45.21	68.48	81.16	93.75
3	21.42	38.96	59.40	70.62	86.85
4	17.05	35.50	53.93	66.28	78.63
Diclofenac sodium	5.25	10.86	14.95	21.26	76.46

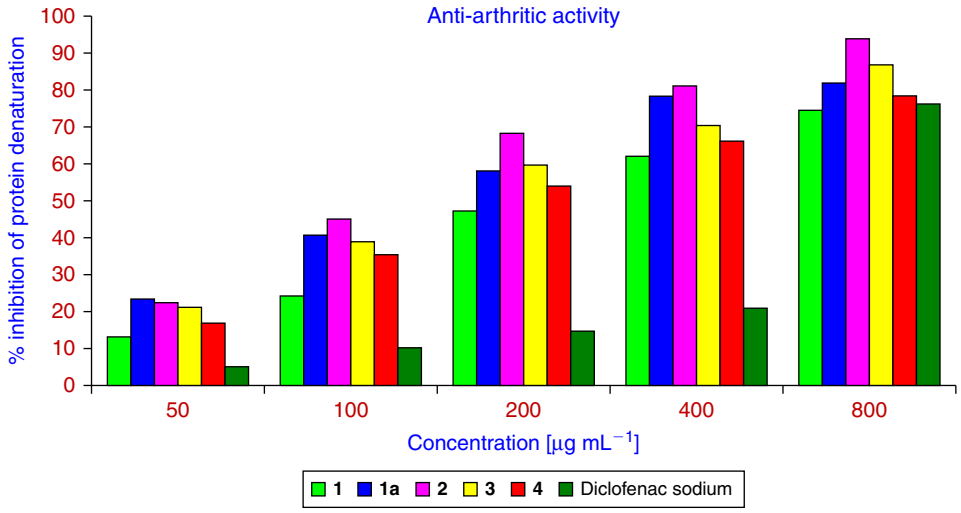


Fig. 2. Anti-arthritic activity of *N*-tosyl tetraaza and diaza cyclophanes **1**–**4**.

Table 2. In vitro anti-inflammatory activity of *N*-tosyl tetraaza and diaza cyclophanes 1–4 by HRBC membrane stabilization

Each value represents mean ± s.d. of three observations

Cyclophane sulfonamide	Activity (% prevention of lysis)			
	10 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}
1	39.63 ± 0.54	53.38 ± 0.31	73.11 ± 0.63	85.63 ± 0.25
1a	34.18 ± 0.35	47.01 ± 0.56	61.36 ± 0.72	74.54 ± 0.48
2	57.38 ± 0.46	72.99 ± 0.07	89.65 ± 0.37	96.88 ± 0.26
3	43.20 ± 0.71	51.97 ± 0.83	74.79 ± 0.52	82.34 ± 0.35
4	54.23 ± 0.35	65.93 ± 0.48	89.73 ± 0.85	97.23 ± 0.40
Prednisolone	46.03 ± 0.13	57.94 ± 0.39	84.87 ± 0.36	91.01 ± 0.45

cyclophanes on BSA is owing to the increased hydrophobicity compared with diclofenac sodium. The degree of anti-arthritic activity of cyclophane amides are **2** > **3** > **1a** > **4** > **1** at 800 μg mL^{-1} is found to be 93.75, 86.85, 82.02, 78.63 and 74.42 %, respectively (Fig. 2).

In vitro anti-inflammatory activity was studied by the human red blood cells (HRBC) membrane stabilization method.[29] 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. Since the HRBC membrane are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the drug. The observed data showing the anti-inflammatory activity of the compounds and the control drug are given in Table 2 and Fig. 3. Further, in the present study the anti-inflammatory activity of compounds **1**, **1a**, **2**, **3** and **4** was investigated using HRBC membrane stabilization and with prednisolone as the standard, the results of which are shown in Table 2.

The anti-inflammatory activities of all the *N*-tosyl tetraaza and diaza cyclophanes are concentration dependent. At the higher concentration, the aza cyclophanes **1a**, **2**, **3** and **4** exhibited better anti-inflammatory activity than at lower

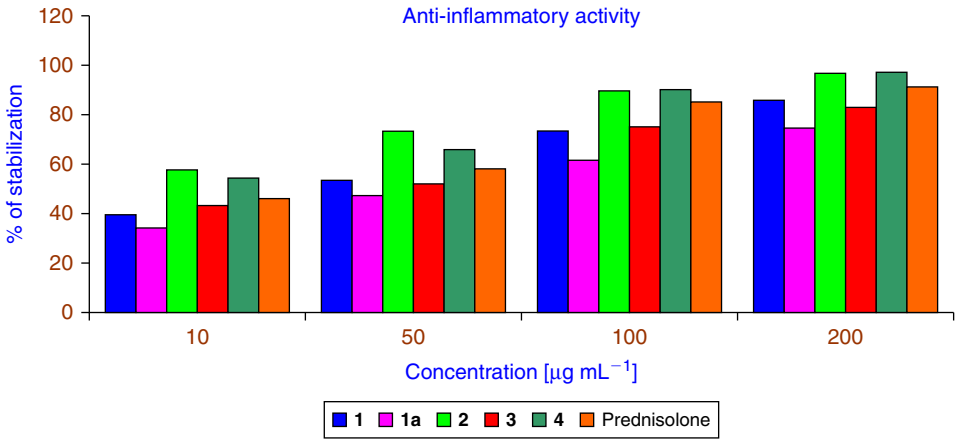


Fig. 3. Anti-inflammatory activity of *N*-tosyl tetraaza and diaza cyclophanes **1**–**4**.

concentration. The sulfonamide macrocycles **4** and **2** were found to possess the maximum anti-inflammatory activity (97.23 and 96.88 % at 200 μg mL^{-1}, respectively) when compared with the reference drug prednisolone (91.01 % at 200 μg mL^{-1}), which clearly shows that anti-inflammatory activity of synthesized cyclophanes is superior to the reference drug. The better anti-inflammatory activity of sulfonamide macrocycles could be owing to their binding to the erythrocyte membranes with subsequent alteration of the charges on the membrane surface of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges that are involved in the haemolysis of red blood cells. The degree of anti-inflammatory activity of *N*-tosyl cyclophane amines **4** > **2** > **1** > **3** > **1a** at 200 μg mL^{-1} is found to be 97.23, 96.88, 85.63, 82.34 and 74.54 %, respectively (Fig. 3).

Conclusion

In conclusion, all the cyclophanes **1**, **1a**, **2**, **3** and **4** show superior anti-arthritic activity than the reference drug diclofenac sodium. However, the *N*-tosyl tetraaza cyclophanes **4** and **2** show superior anti-inflammatory activity at lower concentration (200 μg mL^{-1}) than the reference drug prednisolone, which could lead to their development as anti-inflammatory drugs (NSAIDs). Further studies are required to determine their toxicity, bioavailability, mode of action etc. The syntheses of similar *N*-tosyl tetraaza cyclophanes with different biologically important spacer units to improve the solubility and efficacy, in vivo anti-arthritic, anti-inflammatory assay and molecular recognition towards various biologically important anions are under investigation.

Experimental

General

All reagents and solvents employed were of the best grade available and were used without further purification. The melting points were determined using a Mettler Toledo melting point apparatus by the 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. Elemental analyses for the compounds were carried out using the Elementar Vario EL III elemental analyzer. Pre-coated silica gel plates from Merck were used for TLC analysis. Column chromatography was carried out using silica gel (100–200 mesh) purchased from ACME.

*Procedure for the Synthesis of Precyclophane (**6**)*

A solution of *p*-toluenesulfonyl chloride (29.2 mmol) in dry chloroform (50 mL) was added dropwise to a well-stirred solution of *m*-terphenyl diamine **5** (13.9 mmol) and pyridine (30.2 mmol) in dry chloroform (150 mL) at 0–5°C. The resulting yellow suspension was stirred for 5 h after which time the solvent was removed under reduced pressure. The obtained solid was washed well with water to remove pyridine hydrochloride and then purified by column chromatography (SiO_2) with chloroform as the eluting solvent. Yield 85 %; mp 205°C. ν_{max} (KBr)/cm^{-1} 1598, 1567. δ_H (400 MHz, $CDCl_3$) 7.79 (d, *J* 8.1, 4H), 7.70 (s, 1H), 7.54 (t, *J* 8.1, 5H), 7.28–7.32 (m, 9H), 7.19 (d, *J* 7.5 Hz, 1H), 4.63 (t, *J* 3.4, 2H), 4.19, 4.20 (dd, *J* 3.5, 3.4, 4H), 2.44 (s, 6H). δ_C (100 MHz, $CDCl_3$) 142.8, 140.9, 139.1, 137.9, 137.3, 130.1, 129.6, 128.7, 128.0, 127.6, 127.1, 126.6, 48.4, 21.6. *m/z* (ES) 614.1 [$(M+NH_4)^+$].

General Procedure for the Synthesis of N*-Tosyl Tetraaza and Diaza Cyclophanes (**1**–**4**)*

A solution of *N*-tosyl *m*-terphenyl diamine **6** (8.0 mmol) in dry acetonitrile (125 mL) and a solution of the corresponding *o*-xylylene dibromide, *m*-xylylene dibromide, *m*-nitro xylylene dibromide, 2,6-(bisbromomethyl)pyridine or *m*-terphenyl dibromide (8.4 mmol) in dry acetonitrile (125 mL) were simultaneously added dropwise to a well-stirred mixture of anhydrous potassium carbonate (80 mmol) in dry acetonitrile (250 mL) for 8 h. After the addition was complete, the reaction mixture was stirred for a further 48 h. The reaction mixture was filtered and the solvent removed under reduced pressure to give a solid, which was washed with water (2 × 100 mL) and purified by column chromatography (SiO_2) with chloroform as the eluting solvent.

*5,9,15,19-*N*-p-Toluenesulfonyltetraaza 1,3,11,13(1,4),2,12(1,3),7,17(1,2) octabenzenacycloeicosaphane (**1**)*

Yield 68 %, mp 252°C. ν_{max} (KBr)/cm^{-1} 1598, 1597. δ_H (400 MHz, $CDCl_3$) 7.67 (d, *J* 8.2, 8H), 7.46 (s, 2H),

7.37 (d, *J* 8.2, 2H), 7.31 (d, *J* 8.1, 4H), 7.27 (d, merged with $CHCl_3$, *J* 8.1, 8H), 7.19 (d, *J* 8.2, 8H), 7.02 (d, *J* 8.1, 8H), 6.83 (ABq, *J* 8.2, 8H), 4.19 (s, 16H), 2.44 (s, 12H). δ_C (100 MHz, DMSO-d_6) 158.2, 140.8, 139.3, 138.7, 137.2, 136.6, 131.3, 129.5, 128.9, 128.3, 127.9, 127.2, 124.8, 50.9, 48.2, 21.4. *m/z* (ES) 1414.9 $[(M+NH_4)^+]$. Anal. Calc. for $C_{84}H_{76}N_4O_8S_4$: C 72.18, H 5.48, N 4.01. Found: C 72.46, H 5.57, N 4.08 %.

*5,9,15,19-*N-p-*Toluenesulfonyltetraaza1,3,11,13(1,4),2,7,12,17(1,3)octabenzenacycloeicosaphane (**1a**)*

Yield 68 %, mp 267°C. ν_{max} (KBr)/cm^{-1} 1597, 1516. δ_H (400 MHz, $CDCl_3$) 7.75 (d, *J* 8.0, 8H), 7.40 (d, *J* 8.1, 8H), 7.35 (d, *J* 8.0, 8H), 7.25 (d, merged with $CHCl_3$, *J* 8.0, 4H), 7.09 (d, *J* 7.3, 2H), 7.05 (s, 4H), 6.99 (d, *J* 7.3, 4H), 6.95 (d, *J* 8.0, 8H), 6.68 (d, *J* 8.2, 2H), 4.18 (s, 16H), 2.45 (s, 12H); δ_C (100 MHz, DMSO-d_6) 158.3, 140.9, 139.2, 138.8, 137.6, 137.0, 136.7, 129.6, 129.1, 128.7, 128.2, 127.7, 127.1, 124.9, 51.1, 50.7, 21.6. *m/z* (ES) 1414.6 $[(M+NH_4)^+]$. Anal. Calc. for $C_{84}H_{76}N_4O_8S_4$: C 72.18, H 5.48, N 4.01. Found: C 72.45, H 5.56, N 4.07 %.

*5,9,15,19-*N-p-*Toluenesulfonyltetraaza1,3,11,13(1,4),2,7,12,17(1,3)phane7,17-bis(*m*-nitrobenzeno)hexabenzenacycloeicosaphane (**2**)*

Yield 50 %, mp 163°C. ν_{max} (KBr)/cm^{-1} 1597, 1533. δ_H (400 MHz, $CDCl_3$) 7.78 (d, *J* 7.8, 8H), 7.61 (d, *J* 7.6, 4H), 7.36–7.50 (m, 16H), 7.27 (d, merged with $CHCl_3$, *J* 8.2, 2H), 7.22 (s, 4H), 7.09 (d, *J* 7.8, 4H), 7.02 (d, *J* 7.8, 4H), 6.98 (d, *J* 7.8, 4H), 4.25 (s, 16H), 2.47 (s, 12H). δ_C (100 MHz, DMSO-d_6) 158.5, 141.2, 139.4, 138.9, 137.7, 137.1, 136.6, 129.6, 129.1, 128.7, 128.3, 127.6, 127.2, 124.8, 50.8, 50.1, 21.9. *m/z* (ES) 1504.6 $[(M+NH_4)^+]$. Anal. Calc. for $C_{84}H_{74}N_6O_{12}S_4$: C 67.81, H 5.01, N 5.65. Found: C 67.98, H 5.09, N 5.72 %.

*5,9,15,19-*N-p-*Toluenesulfonyltetraaza1,3,11,13(1,4),2,7,12,17(1,3),7,17-dipyridinohexabenzenacycloeicosaphane (**3**)*

Yield 40 %, mp 135°C. ν_{max} (KBr)/cm^{-1} 1596, 1516. δ_H (400 MHz, $CDCl_3$) 7.70 (d, *J* 8.1, 8H), 7.48 (d, *J* 7.8, 2H), 7.42 (d, *J* 8.1, 2H), 7.36 (d, *J* 8.1, 8H), 7.29 (d, *J* 8.1, 8H), 7.24 (d, *J* 8.1, 8H), 7.13 (d, *J* 7.8, 4H), 7.09 (d, *J* 8.1, 4H), 6.98 (d, *J* 8.1, 4H), 4.25 (s, 16H), 2.42 (s, 12H). δ_C (100 MHz, $CDCl_3$) 155.8, 144.8, 141.3, 140.6, 137.1, 134.9, 129.9, 129.2, 128.7, 128.4, 127.5, 127.1, 126.2, 125.6, 121.8, 52.7, 51.3, 21.7. *m/z* (ES) 1416.5 $[(M+NH_4)^+]$. Anal. Calc. for $C_{82}H_{74}N_6O_8S_5$: C 70.36, H 5.33, N 6.00 %. Found: C 70.50, H 5.41, N 6.08 %.

*5,11-*N-p-*Toluenesulfonyldiaza1,3,7,9(1,4),2,8(1,3)hexabenzenacyclododecaphane (**4**)*

Yield 80 %, mp 261°C. ν_{max} (KBr)/cm^{-1} 1598, 1583, 1518. δ_H (400 MHz, $CDCl_3$) 7.85 (d, *J* 8.2, 4H), 7.42 (d, *J* 8.1, 6H), 7.34 (d, *J* 0.9, 6H), 7.27 (d, merged with $CHCl_3$, *J* 8.1, 8H), 7.14 (d, *J* 8.1, 8H), 4.36 (s, 8H), 2.50 (s, 6H). δ_C (100 MHz, $CDCl_3$) 143.5, 139.3, 137.7, 137.2, 135.5, 129.9, 129.4, 128.7, 128.4, 127.9, 126.9, 126.3, 50.8, 21.5. *m/z* (ES) 851.3 $[(M+H)^+]$. Anal. Calc. for $C_{54}H_{46}N_2O_4S_2$: C 76.21, H 5.45, N 3.29 %. Found: C 76.44, H 5.52, N 3.35 %.

In Vitro Anti-Arthritic Studies

The test solution (0.5 mL) consists of BSA (0.45 mL, 5 % *w/v* aqueous solution) and a solution of aza cyclophane (800, 400, 200, 100, 50 µg in 0.05 mL DMSO). The test control solution (0.5 mL) consists of BSA (0.45 mL, 5 % *w/v* aqueous solution) and distilled water (0.05 mL). The product control solution (0.5 mL) consists of distilled water (0.45 mL) and aza cyclophane solutions (800, 400, 200, 100, 50 µg in 0.05 mL DMSO). The standard solution (0.5 mL) consists of BSA (0.45 mL, 5 % *w/v* aqueous solution) and diclofenac sodium (800, 400, 200 µg mL^{-1} in 0.05 mL water). All of the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, phosphate buffer (2.5 mL) was added to the above solutions. The absorbance was measured using a Systronic UV-Vis Spectrophotometer 118 at 416 nm. The percentage inhibition of protein denaturation can be calculated as:

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

In Vitro Anti-Inflammatory Studies

The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity using the drug prednisolone as a standard. Blood was collected from three healthy volunteers and the collected blood was mixed with an 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 contain the aza cyclophanes dissolved in DMSO (200, 400 and 800 µg mL^{-1}), 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^{-1} 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 haemoglobin content in the supernatant solution was estimated using a Systronic UV-Vis Spectrophotometer 118 at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water 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 produces a variety of disorders. This extracellular activity of this enzyme is related to acute or chronic inflammation. Since the HRBC membrane are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the drug.

Acknowledgements

The authors thank DST, New Delhi, for financial assistance, RSIC, CDRI, Lucknow for MS, DST-FIST for providing the NMR facility for the

Department of Organic Chemistry, University of Madras and Prof. D. Chamundeeswari, Ph. D., Department of Pharmacognazy, Sri Ramachandra College of Pharmacy, Sri Ramachandra University, Chennai-600 116, for in vitro studies. R.P. thanks Orchid Chemicals and Pharmaceuticals Ltd, for providing the laboratory and analytical facility.

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