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Short communication

Synthesis of novel carbazole based macrocyclic amides as potential antimicrobial agents

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ABSTRACT

A series of carbazole based macrocyclic diamides with thia and oxy linkages have been synthesized and the inhibitory activity of the cyclophane amides against human pathogenic bacteria and plant pathogenic fungi are documented. (*S*)-1,10-Bi-2-naphthol [(*S*)-BINOL] based chiral carbazolophane amide emerged as the most interesting compound in this series exhibiting excellent antibacterial and antifungal activities.

1. Introduction

Synthesis of new supramolecules architecturally novel and of potential importance in the context of designing simple models for studying biomolecular interactions stimulates the imaginative skill of synthetic chemists. Replacement of the oxygen donor atom in the crown ether with sulfur and/or nitrogen atom and by introducing functional groups viz., amide, ester in the ring would make them as models of protein–metal binding sites in biological systems [1–3]. Synthetic development of new macrocyclic peptide antibiotics against bacteria has undergone dramatic changes during the last few years including biphenomycin B [4], vancomycine type glycopeptide antibiotics [5] and 1,1′-binaphthyl carbazole linked cyclic peptides [6,7]. Cyclic peptides with open pores are useful as transport vehicles for biologically important ions and neutral molecules [8]. Synthetic biphenyl based cyclic amides have been reported for anion complexation [9], and cyclic tetraamide receptors having barbiturate binding domain have also been reported [10]. Supramolecular amides are also used as molecular receptors [11] and in molecular recognition [12] of biologically interacting substrates including anti-HIV active macrocyclic amides [13]. Copper complexes of macrocyclic compounds exhibit increased antibacterial and antifungal activities than the uncomplexed macrocyclic compounds [14]. We have recently reported the synthesis of permanent fluorescence sensing chiral fluorophoric macrocycles with antibacterial activity [15] and cyclophane amides with anti-inflammatory activity [16]. However, to the best of our knowledge, no carbazole based amide macrocycles have been reported. Herein, we report the synthesis of carbazolophane amides **1**–**6** along with their antibacterial and antifungal activities.

2. Chemistry

Six new carbazole based macrocyclic amides (**1**–**6**) were synthesized, which include possible combinations of benzene, pyridine, biphenyl and chiral binaphthyl unit (Schemes 1–4). One equivalent of isophthaloyl dichloride (**12**), pyridine 2,6-dicarboxylicacid chloride (**13**), biphenicacid chloride (**14**), and the extended diacid chlorides **15**, **19** and **23** react with 1 equiv. of thia bridged carbazole diamine **11** (prepared by the reaction of 3,6-bis (bromomethyl)-9-ethylcarbazole (**10**) [17] with 2-aminothiophenol in the presence of KOH) to give the carbazolophane amides **1**–**6** in 27–40% yield and the rest of the yield contained unidentified products. All the new compounds gave satisfactory IR, ^{1}H and ^{13}C NMR, mass spectral and elemental analysis.

3. Biology

Antimicrobial activities of compounds were tested using disc diffusion assay [18]. Tested microorganism strains: human pathogenic bacteria such as *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Salmonella typhi*; plant pathogenic fungi such as *Rhizoctonia solani*, *Macrophomina phaseolina*, *Curvularia lunata* and

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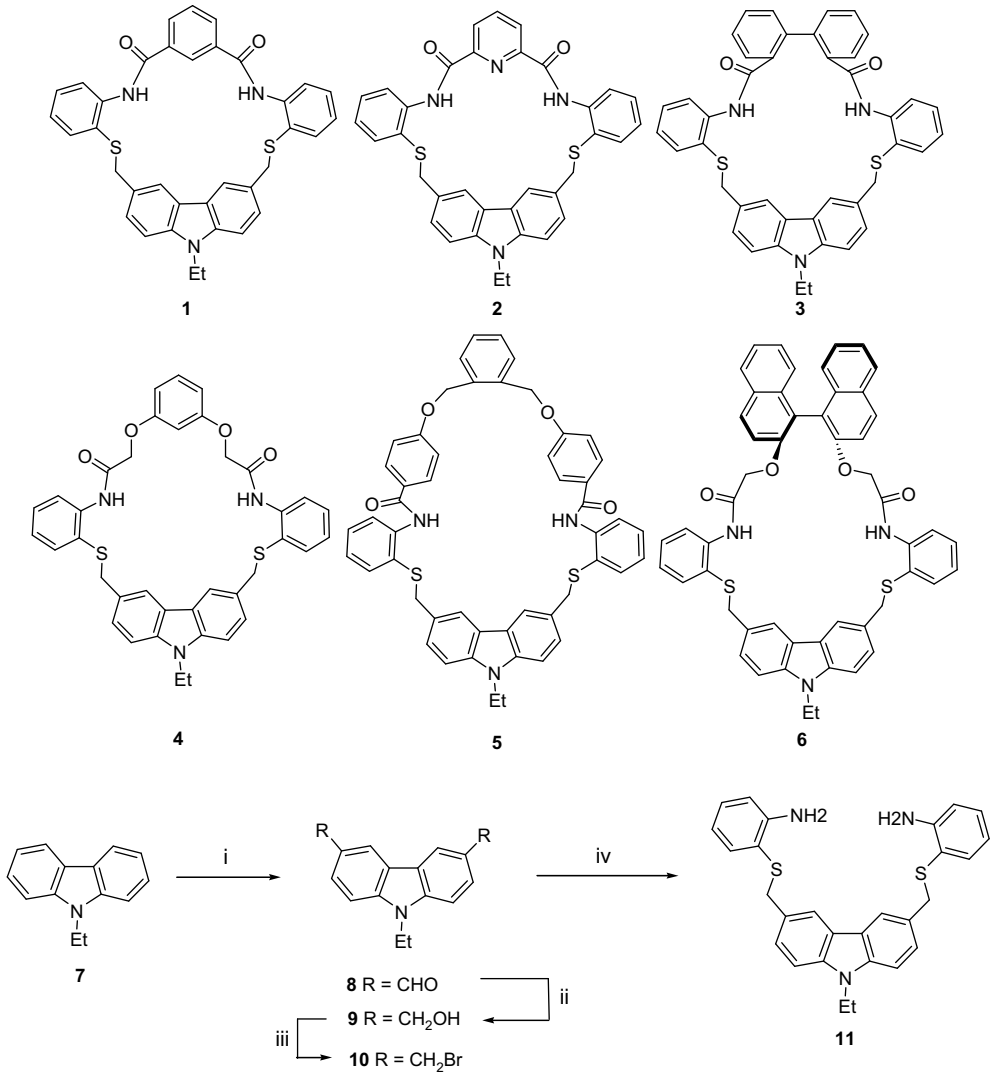
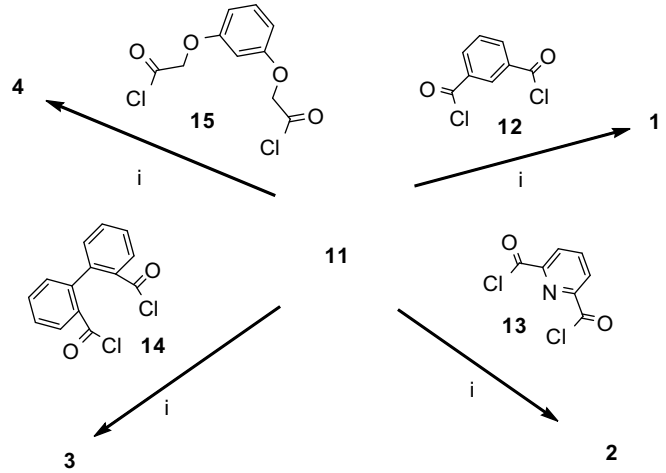


Scheme 1. Reagents and conditions: (i) $POCl_3$/DMF, DCE, reflux, 48 h, **8** (55%); (ii) $NaBH_4$, THF–EtOH (1:1), rt, 12 h, **9** (80%); (iii) 48% HBr (aq.), 0 °C, 5 h, **10** (72%); (iv) 2-aminothiophenol, KOH, TBAB, toluene–H_2O (1:1), reflux, 5 h, **11** (80%).

Alternaria alternata (obtained from the culture collections of Biocontrol and Microbial Metabolites Lab, Center for Advanced Studies in Botany, University of Madras, Chennai, India) under in vitro conditions. The observed data on the antimicrobial activity of the compounds and control drugs are given in Tables 1 and 2 in the form of minimum inhibitory concentration (MIC).

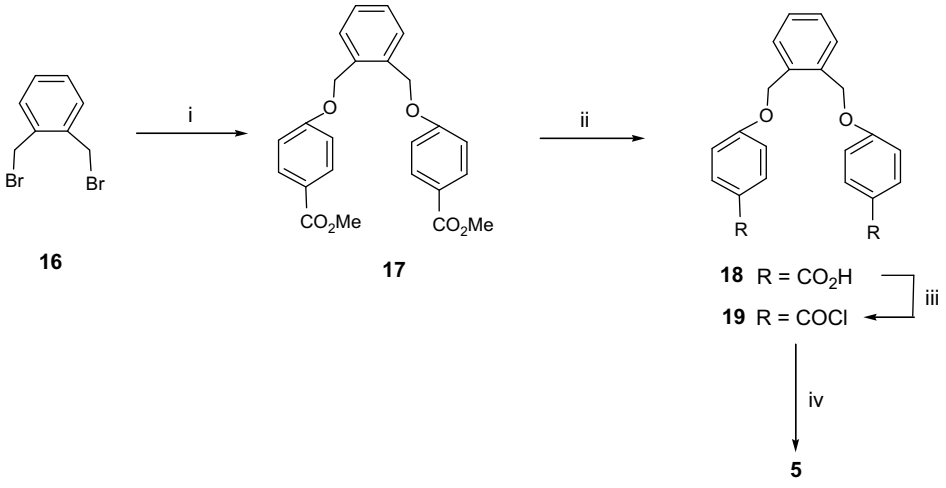


Scheme 2. Reagents and conditions: (i) TEA, $CHCl_3$ (dry), 12 h, **1** (40%), **2** (32%), **3** (28%), **4** (37%).

4. Results and discussion

The precursor diamine **11** for the synthesis of cyclophane amides **1**–**6** was synthesized as follows. Diamine **11** was prepared in 80% yield by the reaction of 3,6-bis(bromomethyl)-9-ethylcarbazole (**10**) [17] with 2-aminothiophenol in the presence of KOH and TBAB as phase transfer catalyst in toluene–water (1:1) system under refluxing conditions. The dibromide **10** was obtained from the diol **9** using aq. HBr at 0 °C. The dialcohol **9** in turn was obtained in 80% yield by the reduction of the dialdehyde **8** with $NaBH_4$ in THF–EtOH (1:1) mixture. Formylation of *N*-ethylcarbazole (**7**) with DMF and $POCl_3$ in 1,2-dichloroethane afforded the dialdehyde **8** after purification by column chromatography using SiO_2 in 55% yield (Scheme 1).

In order to test the synthetic utility of precyclophane **11** for the synthesis of amide carbazolophanes, 1 equiv. of precyclophane **11** was coupled with 1 equiv. of isophthaloyl dichloride (**12**), pyridine 2,6-dicarboxylicacid chloride (**13**), biphenicacid chloride (**14**) and diacid chloride **15** [19] (prepared from corresponding diacid) to give the carbazolophane amides **1**, **2**, **3** and **4** in 40%, 32%, 28%, and 37% yields, respectively, after purification by column



Scheme 3. Reagents and conditions: (i) *p*-hydroxymethylbenzoate, K_2CO_3, KI, CH_3CN, reflux, 12 h, **17** (92%); (ii) 25% KOH, MeOH, reflux, **18** (85%); (iii) $SOCl_2$, TEA, DCM, reflux, 3 h, **19** (82%); (iv) **11** (1 equiv.), TEA, $CHCl_3$ (dry), rt, 12 h, **5** (27%).

chromatography and the rest of the yield contained unidentified products (Scheme 2). The structure of cyclophanes **1**–**4** was confirmed from spectroscopic and analytical data.

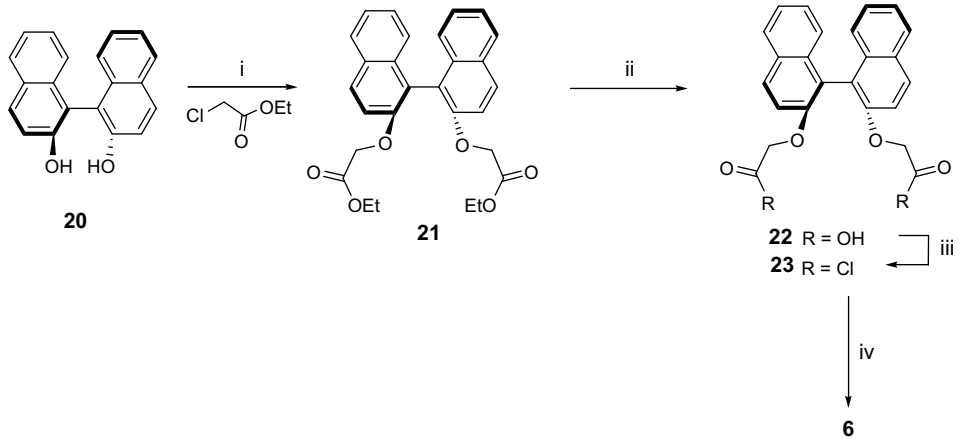
^{1}H NMR spectrum of carbazolophane amide **1** displayed a three proton triplet at δ 1.28 ($J = 7.2$ Hz) for methyl proton present in the *N*-ethyl moiety, and singlet at δ 3.94 for SCH_2 protons. A two proton quartet at δ 4.01 ($J = 7.2$ Hz) for methylene protons of the *N*-ethyl unit, and a two proton singlet at δ 8.97 for amide NH protons appeared in addition to the aromatic protons. ^{13}C NMR spectrum of **1** showed a peak at δ 13.9 and δ 37.5 for methyl and methylene carbon of ethyl unit, respectively. The SCH_2 carbons appeared at δ 44.2 and a peak at δ 163.0 for carbonyl carbon in addition with 16 aromatic carbons.

Large cavity carbazolophanes with amide linkages using diacid chloride **19** was synthesized as follows. The diacid chloride **19** was obtained by the reaction of *o*-xylene dibromide with 2.1 equiv. *p*-hydroxymethylbenzoate in the presence of K_2CO_3 and KI in dry CH_3CN to give the diester **17** [20] in 92% yield, which on hydrolysis gave the corresponding diacid **18**. Diacid **18** was then smoothly converted into the diacid chloride **19** in 82% yield by using thionyl chloride. Reaction of 1 equiv. diamine **11** with 1 equiv. of diacid chloride **19** in the presence of 2.1 equiv. of triethylamine in $CHCl_3$ for 12 h at room temperature gave cyclophane amide **5** in 27% yield and the remaining are unidentified products (Scheme 3).

^{1}H NMR spectrum of carbazolophane amide **5** displayed a three proton triplet at δ 1.47 ($J = 7.2$ Hz) and SCH_2 protons appeared as a singlet at δ 4.20. The NCH_2 and OCH_2 protons appeared as a quartet at δ 4.42 ($J = 7.2$ Hz) and as a singlet at δ 4.89, respectively. A two proton singlet at δ 9.01 for amide NH protons appeared in addition to 26 aromatic protons. In ^{13}C NMR spectrum methyl and methylene carbon of *N*-ethyl moiety appeared at δ 12.5, and δ 36.8, respectively. The SCH_2 and OCH_2 methylene carbons appeared at δ 41.6 and δ 64.5 and carbonyl carbon appeared at δ 160.0 in addition to aromatic carbons.

Introduction of optically active (*S*)-1,10-bi-2-naphthol [(*S*)-BINOL] spacer into such cyclophane amides would give chiral carbazolophane. Modifications on compounds with bridged binaphthyl diether linkage have been previously studied [21].

In order to explore the utility of the diamine **11** for the synthesis of chiral carbazolophane amide, the diamine **11** was stirred with 1 equiv. (*S*)-1,10-bi-2-naphthol [(*S*)-BINOL] based chiral diacid chloride **23** in the presence of triethylamine in dry $CHCl_3$ to give the chiral carbazolophane amide **6** in 32% yield and the remaining are unidentified products. The chiral diacid **22** was obtained by the reaction of (*S*)-1,10-bi-2-naphthol [(*S*)-BINOL] with 2.1 equiv. of



Scheme 4. Reagents and conditions: (i) K_2CO_3, KI, CH_3CN, reflux, 12 h, **21** (82%); (ii) 25% KOH, MeOH, reflux, **22** (55%); (iii) $SOCl_2$, TEA, DCM, reflux, 4 h, **20** (63%); (iv) **11**, TEA, $CHCl_3$ (dry), rt, 12 h, **6** (32%).

Table 1
In vitro antibacterial activity against human pathogens (minimum inhibitory concentration in μg/ml) for amide carbazolophanes

Carbazolophane amides	*P. mirabilis*	*P. vulgaris*	*S. aureus*	*S. typhi*
1	50	60	20	50
2	5	30	10	30
3	40	40	70	60
4	20	60	40	30
5	10	40	30	20
6	5	5	10	10
Tetracycline	20	35	20	15
Control	NI	NI	NI	NI

NI: no inhibition.

ethyl chloroacetate in the presence of potassium carbonate followed by the hydrolysis of the resulting chiral diethylester **21** to give the corresponding diacid **22**, which was then reacted with thionyl chloride to give diacid chloride **23** (Scheme 4).

^{1}H NMR spectrum of carbazolophane **6** displayed a three proton triplet and two proton quartet at δ 1.15 ($J = 7.2$ Hz) and δ 4.10 ($J = 7.2$ Hz) for methyl and methylene proton of *N*-ethyl unit, respectively. The SCH_2 and OCH_2 protons attached to the (*S*)-1,10-bi-2-naphthol [(*S*)-BINOL] unit as well as those attached to the carbazole unit appeared as two doublets at δ 3.65 (d, 2H, $J = 12.6$ Hz), 3.78 (d, 2H, $J = 12.3$ Hz) and 4.20 (d, 2H, $J = 15$ Hz), 4.45 (d, 2H, $J = 15$ Hz) due to the atropisomerism of the (*S*)-1,10-bi-2-naphthol [(*S*)-BINOL] unit, in addition to the aromatic protons. In ^{13}C NMR spectrum *N*-ethyl unit of methyl and methylene carbon appeared at δ 17.0, and δ 37.5, respectively. The SCH_2 and OCH_2 carbons appeared at δ 42.1 and δ 67.3 and carbonyl carbon appeared at δ 165.5 in addition to aromatic carbons.

In the present study, antimicrobial activities of six different newly synthesized amide carbazolophanes were evaluated against four human pathogenic bacteria such as *P. mirabilis*, *P. vulgaris*, *S. aureus*, and *S. typhi*. Antifungal activity of these compounds was also tested against four plant pathogenic fungi viz., *R. solani*, *M. phaseolina*, *C. lunata* and *A. alternata* under in vitro conditions. The biological screening results of carbazolophane amides with 10% DMSO as control and with commercial antibiotics viz., tetracycline (for human pathogenic bacteria), carbendazim (for plant pathogenic fungi) are tabulated (Tables 1 and 2) in the form of minimum inhibitory concentration (MIC).

4.1. Effect of carbazolophane amides on the growth of human pathogens

All the six cyclic carbazole amides exhibited different levels of antibacterial activity against the four-tested human pathogenic bacteria compared to DMSO as control. Further, the antibacterial activity of the test compounds was dose dependent and was remarkable at higher concentrations. The minimum inhibitory concentration (MIC) of carbazolophane amides **1**–**6** against bacterial human pathogens as determined by well diffusion method ranged between 5 and 80 μg/ml. The chiral carbazolophane amide **6** shows remarkable antibacterial activity against tested pathogens namely, *P. mirabilis*, *P. vulgaris*, *S. aureus* and *S. typhi* compared to rest of the cyclophane amides and commercial antibiotic, tetracycline at lowest concentration ranging from 5 to 10 μg/ml, followed by cyclophanes **2** and **5** (ranging from 5 to 30 μg/ml and 10–40 μg/ml, respectively) compared with reference control. The minimum inhibitory concentration (MIC) values are relatively low for carbazolophane amides **1**, **3**, **4** and **5** as compared with reference control (Table 1).

Table 2
In vitro antifungal activity against plant pathogens (minimum inhibitory concentration in μg/ml) for amide carbazolophanes

Carbazolophane amides	*R. solani*	*M. phaseolina*	*C. lunata*	*A. alternata*
1	30	30	20	15
2	20	10	15	15
3	20	15	20	25
4	50	45	35	25
5	40	50	20	15
6	20	15	10	10
Carbendazim	25	18	15	12
Control	NI	NI	NI	NI

NI: no inhibition.

4.2. Effect of carbazolophane amides on the growth of plant pathogenic fungi

Similar to antibacterial activity, all the six carbazolophane amides exhibited different levels of antifungal activity against the four-tested plant pathogenic fungi compared to DMSO control. Further, the antifungal activity of the test compounds was dose dependent and was remarkable at higher concentrations. The minimum inhibitory concentrations (MICs) of the carbazolophanes **1**–**6** against fungal plant pathogens as determined by well diffusion method ranged between 10 and 45 μg/ml. Among the six cyclophanes tested, chiral cyclophane amide **6** (ranging from 10 to 20 μg/ml) significantly inhibited all the four plant pathogens namely, *R. solani*, *M. phaseolina*, *C. lunata* and *A. alternata* compared to rest of the cyclophanes and commercial fungicide, carbendazim. Carbazolophane **2** was rated as the second best cyclophane amide with antifungal activity (ranging from 10 to 20 μg/ml) compared with reference control. The minimum inhibitory concentration (MIC) values are relatively low for cyclophanes **1**, **3**, **4** and **5** as compared with reference control (Table 2).

The results obtained have clearly indicated that (*S*)-1,10-bi-2-naphthol [(*S*)-BINOL] based chiral carbazolophane **6** acts as an excellent antibacterial and antifungal agent as compared to other cyclophanes. This may be due to the rigid and restricted conformational behavior of macrocyclic carbazole amides in the presence of (*S*)-1,10-bi-2-naphthol [(*S*)-BINOL] unit [22]. In molecular orbital (MO) calculation carried out using MOPAC (PM_3) method on chiral carbazolophane amide **6**, the minimization energy (MM2) of chiral

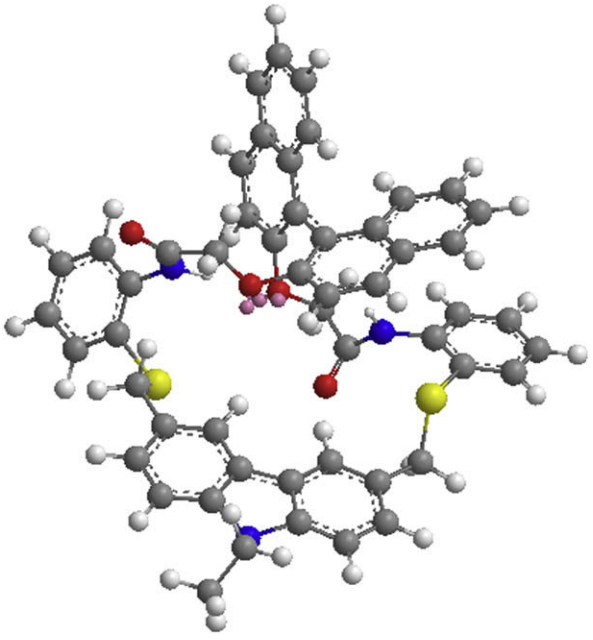


Fig. 1. Energy minimization of cyclophane amide **6**. Heat of formation of cyclophane amide **6** is 5.8242 kcal/mol.

carbazolophane amide was found to be 5.8242 kcal/mol. Fig. 1 shows that molecule has good rigidity and two naphthyl rings are in twisted form.

5. Conclusion

In conclusion, the carbazolophanes **2** and **6** exhibited good antibacterial and antifungal activities against all the four human pathogenic bacteria and plant pathogenic fungi. Chiral carbazolophane **6** may be developed as antimicrobial drug as it showed superior activity against all the tested pathogens than the other compounds including tetracycline and carbendazim. Molecular recognition studies of various biologically important anions with these carbazolophane amides are under investigation.

6. Experimental

6.1. Chemistry

All the reagents and solvents employed were of the best grade available and were used without further purification. The melting points were determined using a Toshniwal melting point apparatus by open capillary tube method and were uncorrected. Spectroscopic data were recorded by the following instruments IR: FTIR-8300 spectrophotometer; NMR Bruker 300 MHz; MS: EI-MS spectra on Jeol DX-303 mass spectrometer and FAB-MS spectra Jeol SX 102/DA-6000 mass spectrometer. The elemental analyses for the compounds were carried out using the Perkin–Elmer 240B elemental analyzer.

6.2. General procedure for the synthesis of carbazolophane amides (*1*–*6*)

A solution of the corresponding diacid chlorides (0.5 mmol) in dry chloroform (100 ml) and a solution of diamine **11** (0.5 mmol) and triethylamine (1.1 mmol) in dry chloroform (100 ml) were simultaneously added drop wise to a well-stirred solution of chloroform (500 ml) for 6 h. After the addition was complete, the reaction mixture was stirred 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 triethylamine hydrochloride and then dried over sodium sulfate. Removal of the chloroform under reduced pressure gave cyclophane as a crude material, which was purified by column chromatography (SiO_2).

6.2.1. Diamine *11*

Yield 80%; mp 114 °C; IR (KBr, cm^{-1}): 3382; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.38 (t, 3H, $J = 7.2$ Hz), 3.50 (br s, 4H), 4.10 (s, 4H), 4.29 (q, 2H, $J = 7.2$ Hz), 6.57–6.71 (m, 4H), 7.07–7.13 (m, 2H), 7.13–7.26 (m, 6H), 7.79 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 37.6, 40.3, 108.3, 114.9, 118.1, 118.5, 120.7, 122.8, 126.8, 128.5, 129.8, 136.3, 139.4, 148.4; MS (EI) m/z: 469 (M^+). Elemental Anal. Calcd for $C_{28}H_{27}N_3S_2$: C, 71.60; H, 5.79; N, 8.95. Found: C, 71.48; H, 5.91; N, 8.82.

6.2.2. Carbazolophane *1*

Yield 40%; mp 243 °C; IR (KBr, cm^{-1}): 3344, 1679, 1577, 1510; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.28 (t, 3H, $J = 7.2$ Hz), 3.94 (s, 4H), 4.01 (q, 2H, $J = 7.2$ Hz), 6.65 (d, 2H, $J = 7.8$ Hz), 6.89–6.93 (m, 3H), 7.12 (t, 2H, $J = 7.5$ Hz), 7.32 (d, 2H, $J = 7.8$ Hz), 7.43 (t, 2H, $J = 7.8$ Hz), 7.58 (s, 2H), 7.67 (s, 1H), 7.73 (d, 2H, $J = 6.9$ Hz), 8.49 (d, 2H, $J = 8.1$ Hz), 8.97 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 37.5, 44.2, 108.3, 119.3, 119.8, 122.7, 124.0, 124.4, 125.4, 126.2, 127.7, 128.6, 130.1, 131.0, 133.6, 137.2, 139.3, 141.2, 163.0; MS (EI) m/z: 599 (M^+). Elemental Anal. Calcd for $C_{36}H_{29}N_3O_2S_2$: C, 72.09; H, 4.87; N, 7.01. Found: C, 72.21; H, 4.73; N, 7.19.

6.2.3. Carbazolophane *2*

Yield 32%; mp 221 °C; IR (KBr, cm^{-1}): 3330, 1672, 1577; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.27 (t, 3H, $J = 7.2$ Hz), 3.96 (s, 4H), 4.00 (q, 2H, $J = 7.2$ Hz), 6.61 (d, 2H, $J = 7.7$ Hz,), 6.83–6.91 (m, 3H), 7.10 (t, 2H, $J = 7.2$ Hz), 7.29 (d, 2H, $J = 7.6$ Hz), 7.39 (t, 2H, $J = 7.8$ Hz), 7.56 (s, 2H), 7.70 (d, 2H, $J = 6.9$ Hz), 8.42 (d, 2H, $J = 8.1$ Hz), 8.94 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.6, 37.4, 44.1, 108.2, 119.1, 119.6, 122.5, 124.0, 124.3, 125.3, 126.0, 127.5, 128.4, 130.0, 131.0, 137.1, 139.1, 141.1, 162.0; MS (EI) m/z: 600 (M^+). Elemental Anal. Calcd for $C_{35}H_{28}N_4O_2S_2$: C, 69.97; H, 4.70; N, 9.33. Found: C, 69.82; H, 4.82; N, 9.49.

6.2.4. Carbazolophane *3*

Yield 28%; mp 252 °C; IR (KBr, cm^{-1}): 3326, 1664, 1566, 1505; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.33 (t, 3H, $J = 7.2$ Hz), 4.16 (s, 4H), 4.23 (q, 2H, $J = 7.2$ Hz), 6.80 (t, 2H, $J = 7.5$ Hz), 6.99–7.06 (m, 6H), 7.11–7.17 (m, 8H), 7.46 (dd, 2H, $J = 7.8$ Hz, $J = 1.2$ Hz), 7.74 (s, 2H), 8.04 (d, 2H, $J = 8.1$ Hz), 9.02 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.7, 37.6, 41.0, 107.7, 120.7, 122.5, 123.1, 125.1, 126.5, 126.9, 127.4, 128.0, 128.7, 129.8, 130.0, 132.7, 136.0, 138.3, 139.4, 139.7, 168.1; MS (EI) m/z: 676 (M^+). Elemental Anal. Calcd for $C_{42}H_{33}N_3O_2S_2$: C, 74.64; H, 4.92; N, 6.22. Found: C, 74.81; H, 4.81; N, 6.35.

6.2.5. Carbazolophane *4*

Yield 37%; mp 248 °C; IR (KBr, cm^{-1}): 3390, 1683, 1581, 1510; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.29 (t, 3H, $J = 7.2$ Hz), 3.93 (s, 4H), 4.13 (s, 4H), 4.19 (q, 2H, $J = 7.2$ Hz), 6.51 (dd, 2H, $J = 8.1$ Hz, $J = 2.4$ Hz), 6.60 (t, 1H, $J = 2.4$ Hz), 6.85 (dd, 2H, $J = 8.4$ Hz, $J = 1.5$ Hz), 7.04–7.13 (m, 5H), 7.33 (td, 2H, $J = 7.8$ Hz, $J = 1.2$ Hz), 7.68 (dd, 2H, $J = 9.3$ Hz, $J = 1.2$ Hz), 7.79 (d, 2H, $J = 1.2$ Hz), 8.37 (dd, 2H, $J = 8.2$ Hz, $J = 1.2$ Hz), 9.60 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.6, 37.6, 42.8, 67.6, 103.5, 107.4, 108.0, 119.5, 120.5, 122.8, 123.0, 124.6, 126.7, 127.0, 130.3, 130.6, 136.5, 139.6, 139.8, 158.6, 165.7; MS (EI) m/z: 660 (M^+). Elemental Anal. Calcd for $C_{38}H_{33}N_3O_4S_2$: C, 69.17; H, 5.04; N, 6.37. Found: C, 69.27; H, 5.18; N, 6.59.

6.2.6. Carbazolophane *5*

Yield 27%; mp 140 °C; IR (KBr, cm^{-1}): 3367, 1676, 1596, 1498; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.47 (t, 3H, $J = 7.2$ Hz), 4.20 (s, 4H), 4.42 (q, 2H, $J = 7.2$ Hz), 4.89 (s, 4H), 6.81 (d, 4H, $J = 8.7$ Hz), 6.92–7.12 (m, 8H), 7.35–7.56 (m, 8H), 7.69–7.86 (m, 4H), 8.00 (s, 2H), 9.01(s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.5, 36.8, 41.7, 64.5, 107.7, 108.0, 113.0, 113. 2, 119.0, 119.3, 121.4, 123.0, 125.1, 125.7, 127.4, 127.6, 127.7, 129.4, 131.2, 132.9, 138.3, 165.5; m/z (FAB-MS): 812 (M^+). Elemental Anal. Calcd for $C_{50}H_{41}N_3O_4S_2$: C, 73.96; H, 5.09; N, 5.17. Found: C, 73.82; H, 5.17; N, 5.28.

6.2.7. Carbazolophane *6*

Yield 32%; mp 110 °C; $[\alpha]^{25}_D$ −112.14, (c 0.01, $CHCl_3$); IR (KBr, cm^{-1}): 3328, 1689, 1583, 1517; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.15 (t, 3H, $J = 7.2$ Hz), 3.65 (d, 2H, $J = 12.6$ Hz), 3.78 (d, 2H, $J = 12.3$ Hz), 4.10 (q, 2H, $J = 7.2$ Hz), 4.20 (d, 2H, $J = 15$ Hz), 4.45 (d, 2H, $J = 15$ Hz), 6.68 (d, 2H, $J = 8.1$ Hz), 6.93 (d, 2H, $J = 8.4$ Hz), 6.99 (t, 2H, J = 7.2 Hz), 7.15 (d, 2H, $J = 7.5$ Hz), 7.20 (d, 2H, $J = 6.0$ Hz), 7.23–7.35 (m, 4H), 7.42 (d, 2H, $J = 9.0$ Hz), 7.51 (d, 2H, $J = 6.9$ Hz), 7.69 (s, 2H), 7.86 (d, 2H, $J = 7.2$ Hz), 8.02 (d, 2H, $J = 8.1$ Hz), 8.07 (d, 2H, $J = 9.0$ Hz), 9.00 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 37.5, 42.1, 67.3, 107.4, 114.8, 119.7, 120.4, 122.6, 122.9, 124.2, 124.7, 125.3, 125.6, 126.7, 127.0, 127.2, 127.8, 128.2, 129.7, 130.4, 130.9, 133.9, 136.0, 139.6, 153.3, 165.5; m/z (QIT-MS): 836 (M^+). Elemental Anal. Calcd for $C_{52}H_{41}N_3O_4S_2$: C, 74.71; H, 4.94; N, 5.03. Found: C, 74.88; H, 5.11; N, 5.17.

6.3. Biological activity

6.3.1. Antibacterial studies

Test organisms and their maintenance: the human pathogenic bacterial cultures such as *P. mirabilis*, *P. vulgaris*, *S. aureus* and *S. typhi*

were obtained from the culture collections of Biocontrol and Microbial Metabolites Lab, Center for Advanced Studies in Botany, University of Madras, Chennai, India and the biological screening of carbazolophane amides was performed there itself. The human bacterial pathogens viz., *P. mirabilis*, *P. vulgaris*, *S. aureus* and *S. typhi* were maintained on nutrient agar (NA) consisting of the following (g/l): beef extract 1.0; yeast extract 2.0; peptone 5.0; NaCl 5.0; agar 15.0; distilled H_2O 1 L; pH 7.2 in slants or petriplates at room temperature (28 ± 2 °C). *Effect of carbazolophane amides on the growth of human pathogens*: the minimal inhibitory concentration (MIC) was determined for compounds **1**–**6** by well diffusion assay [17]. The compound at the concentration range of 5–100 μg/ml in 10% DMSO was used in this study with tetracycline as reference control. The minimum inhibitory concentration (MIC) value was taken as the lowest concentration of compound that showed prominent inhibition of bacterial growth after 24 h of incubation at 37 °C.

6.3.2. Antifungal studies

Test organisms and their maintenance: the plant pathogenic fungi viz., *R. solani*, *M. phaseolina*, *C. lunata* and *A. alternata* were obtained from the culture collections of Biocontrol and Microbial Metabolites Lab, Center for Advanced Studies in Botany, University of Madras, Chennai, India and the biological screening of carbazolophane amides was performed there itself. The plant pathogens viz., *R. solani*, *M. phaseolina*, *C. lunata* and *A. alternata* were maintained on potato dextrose agar (PDA) containing the following (g/l): potato 200 g; dextrose 20 g; agar 15 g; distilled H_2O; 1 L; pH 6.5 in slants or Petriplates at room temperature (28 ± 2 °C). *Effect of carbazolophane amides on the growth of plant pathogenic fungi*: similar to antibacterial activity, the minimum inhibitory concentration (MIC) was determined for cyclic amides **1**–**6** by well diffusion assay. The compound at the concentration range of 5–100 μg/ml in 10% DMSO was used in this study with carbendazim as reference control. Minimum inhibitory concentration (MIC) value was taken as the lowest concentration of compound that showed prominent inhibition of fungal growth after 3 days of incubation at 37 °C.

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References

[1] M.W. Hosseini, J.M. Lehn, S.R. Du, K. Gu, M.P. Mertes, J. Org. Chem. 52 (1987) 1662.
[2] J.M. Lehn, Science (Washington, D.C.) 227 (1985) 849.
[3] P.G. Yohannes, M.P. Mertes, K.B. Mertes, J. Am. Chem. Soc. 107 (1985) 8288.
[4] R. Lëpine, J. Zhu, Org. Lett. 14 (2005) 2981.
[5] K.C. Nicolaou, C.N.C. Boddy, S. Bräse, N. Winssinger, Angew. Chem., Int. Ed. 38 (1999) 2096.
[6] J.B. Bremner, J.A. Coates, D.R. Coghlan, D.M. David, P.A. Keller, S.G. Pyne, New J. Chem. 26 (2002) 1549.
[7] J.B. Bremner, J.A. Coates, P.A. Keller, S.G. Pyne, H.M. Witchard, Synlett (2002) 219–222.
[8] H. Kataoka, T. Katagi, Tetrahedron 43 (1987) 4519.
[9] A.M. Costero, M.J. Banuls, M.J. Aurell, M.D. Ward, S. Argent, Tetrahedron 60 (2004) 9471.
[10] R. Brian, E. Unai, S. Troels, J. Chem. Soc., Perkin Trans. 1 (2002) 1723.
[11] A.H. Christopher, H.P. Duncan, Angew. Chem., Int. Ed. Engl. 31 (1992) 792.
[12] C.S. Kyu, V.E. Donna, F. Erkang, D.H. Andrew, J. Am. Chem. Soc. 113 (1991) 7640.
[13] B.S. Jhaumeer-Laulloo, Asian J. Chem. 12 (2000) 775.
[14] C. Sulekh, T. Sangeetika, T. Shalini, Trans. Metal Chem. 29 (2004) 925.
[15] P. Rajakumar, S. Selvam, V. Shanmugaiah, M. Mathivanan, Bioorg. Med. Chem. Lett. 17 (2007) 5270.
[16] P. Rajakumar, A.M. Abdul Rasheed, A.I. Rabia, D. Chamundeeswari, Bioorg. Med. Chem. Lett. 16 (2006) 6019.
[17] K. Tani, Y. Tohda, K. Hisada, M. Yamamoto, Chem. Lett. (1996) 145.
[18] A. Pandurangan, N. Mathivanan, V.R. Prabavathy, V. Kabilan, V. Murugesan, K. Murugesan, Indian J. Exp. Biol. 33 (1995) 357.
[19] D.M. Purohit, V.H. Shah, Indian J. Chem. 38 (1999) 618.
[20] P. Rajakumar, R. Kanagalatha, Tetrahedron 62 (2006) 9735.
[21] J.J.G.S. Van Es, H.A.M. Biemans, E.W. Meijer, Tetrahedron: Asymmetry 8 (1997) 1825.
[22] P. Rajakumar, M. Srisailas, Tetrahedron 57 (2001) 9749.