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Synthesis, antibacterial and antioxidant properties of novel ethylenoindolophanes – a new class of cyclophanes†

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Synthesis of indole based ethylenophanes has been achieved using direct *N*-arylation followed by McMurry coupling. All the ethylenophanes show antibacterial and antioxidant activity similar to that of commercially available drugs.

Introduction

The indole moiety is a bioactive nucleus[1] present in various natural products.[2] Cyclophanes with indole moieties are called indolophanes,[3] and have received much attention during recent times due to their biological applications. Indole based cyclophanes are infrequently encountered systems,[4] though *N*-arylindole has a key role with interesting biological activities, such as anti-estrogenic,[5] analgesic,[6] antiallergy,[7] cyclooxygenase (COX)-1 inhibitory,[8] neuroleptic,[9] 5-HT6 receptor antagonistic,[10] FTase inhibitor (FTIs),[11] and anti HIV-1 activity.[12] Indole derivatives extracted from marine sponges have been recently reported to show antioxidant behavior.[13,14] Although Ullmann-type coupling of indole with aryl halides to synthesize *N*-arylindole represents a straightforward and inexpensive route,[15] the yields are poor, not reliable, and furthermore many discouraging operational problems such as a high reaction temperature, use of highly polar aprotic solvents, strong base, requirement of a large amount of aryl halides and stoichiometric amounts of copper reagents restrict the application of Ullmann coupling for the synthesis of indolophanes. Therefore, the use of a mild and efficient method for generating the *N*-arylindole moiety from indole is highly desirable.[16] Furthermore, the ring closing step is often crucial[17] in the synthesis of cyclophanes, and various reagents based on transition metals such as samarium,[18] cobalt[19] and low valent titanium have been extensively used for the synthesis of cyclophanes.[20,21] Inter- and intramolecular McMurry coupling for the synthesis of stilbenophanes[22] and indolophanes[23] has been reported from our laboratory. Although the antioxidant properties of carbazole based dendrimers was reported recently from our laboratory,[24] the synthesis and biological applications of bis-indolophanes is still a rare observation. It is important to mention here that the synthesis of indole based ethylenophanes by a direct arylation route is not known so far, and hence we report herein the synthesis and the antibacterial and antioxidant activity of the indole based ethylenophanes **1**, **1a**, **2**, **3** and **4** (Fig. 1).

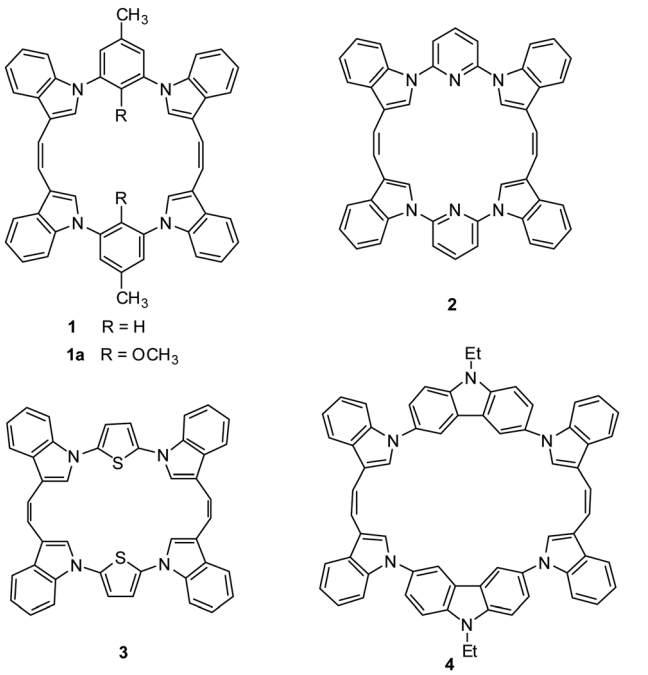

Fig. 1 Structures of the ethylenoindolophanes **1**–**4**.

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The purpose of the synthesis of ethylenophanes **1–4** is twofold. It would be of interest to examine the antioxidant properties of indole based ethylenophanes containing electron donating functionalities and also to examine the antibacterial activity of such ethylenophanes. Hence the antibacterial activity of the ethylenophanes **1–4** was assayed against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Proteus vulgaris* at different concentrations. Similarly, the molecular docking of the indole based ethylenophanes has been carried out using Autodock version 4 with Lamarckian genetic algorithm as a trial study to find the mode of action of such compounds. The ethylenophanes were subjected to docking studies with the bacterial enzyme Topoisomerase IV (PDB ID: 3FV5). The results obtained from the docking studies support the existence of interactions between ethylenophanes and protein. The results are shown in 3D form using Pymol software. Furthermore, the antioxidant behavior of the ethylenophanes **1–4** was determined on the basis of their free radical scavenging activity by a spectroscopic assay method[25] and was measured *in vitro* by using the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH).[26] The antioxidant activity of the ethylenophanes is comparable to that of the standard antioxidant *viz.* ascorbic acid.

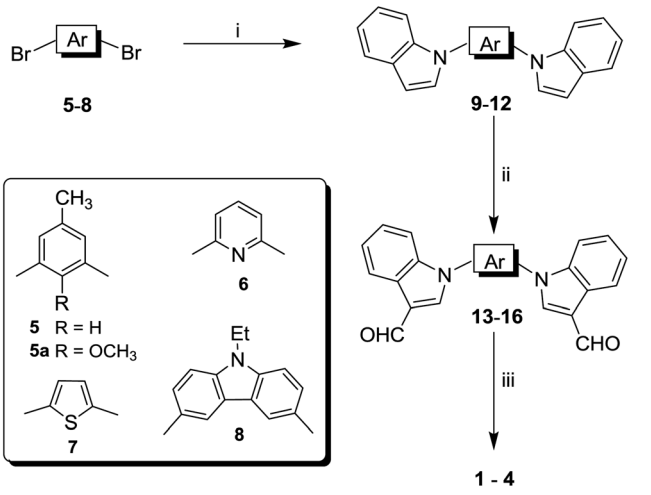
Results and discussion

Synthesis of indole based ethylenophanes **1–4** was achieved by McMurry coupling of the bisindole dialdehyde moiety which, in turn, was obtained from bisindole. The bisindole was obtained by the *N*-arylation of the indole with various dibromides using Buchwald's procedure.[27] The McMurry coupling in the current study leads to the reductive coupling of two carbonyl groups to form a macrocycle by an intermolecular dimerization rather than an intramolecular cyclization process.

The *N*-arylation of 1.0 equiv. dibromides **5–8** with 2.0 equiv. of indole in the presence of CuI, *trans*-1,2-diaminocyclohexane and K_3PO_4 in toluene for 24 h under refluxing conditions afforded the bisindoles **9–12** in 64–85% yields. The ^{1}H NMR spectrum of the bisindole **9** showed a three proton singlet at δ 2.52 for the methyl group attached to the benzene ring, and a set of two proton doublets at δ 7.63 ($J = 8.1$ Hz) and 7.69 ($J = 7.5$ Hz) for the protons at the C_4 and C_2 positions of the indole moiety respectively, and the proton at C_3 of the indole moiety appeared as a doublet at δ 7.36 in addition to the other aromatic proton. In the ^{13}C NMR spectrum, bisindole **9** showed the methyl carbon attached to the benzene ring at δ 21.6 and the C_3 carbon of the indole moiety at δ 104.1 in addition to eleven aromatic signals. Furthermore, the structure of the bisindole **9** was confirmed from elemental analysis and by the appearance of the molecular ion peak at *m*/*z* 322 in the mass spectrum (EI). Similarly the structures of the bisindoles **9a–12** were also confirmed from spectroscopic and elemental analysis.

Vilsmeier formylation of the bisindoles **9–12** with DMF and $POCl_3$ at 0 °C to RT afforded the dialdehydes **13–16** in 72% to 84% yields. The ^{1}H NMR spectrum of the dialdehyde **13** showed a three proton singlet at δ 2.61 for the methyl group attached to the benzene ring, and a singlet at δ 10.10 for the aldehydic protons in addition to other aromatic protons. In the ^{13}C NMR spectrum, dialdehyde **13** showed the methyl carbon attached to the benzene ring at δ 21.63 and the aldehydic carbonyl at δ 184.90 in addition to twelve aromatic signals. The structure of the dialdehyde **13** was confirmed from mass spectral and elemental analysis. Similarly, the structure of the dialdehydes **13a–16** was also confirmed from spectroscopic and elemental analysis.

The dialdehydes **13–16**, on treatment with $TiCl_4$ (20 equiv.) and Zn (40 equiv.) in dry THF under refluxing conditions for 12 h, gave the directly linked ethylenoindolophanes **1**, **1a**, **2**, **3** and **4** in 17%, 18%, 15%, 20% and 15% yields respectively (Scheme 1). The ^{1}H NMR spectrum of the indolophane **1** showed a six proton singlet at δ 2.41 for the methyl group attached to the benzene ring, a four proton singlet at δ 6.84 for the olefinic proton and a two proton singlet at δ 6.53 for inner protons of the benzene ring in addition to other aromatic protons. In the ^{13}C NMR spectrum, the indolophane **1** showed the methyl carbon at δ 21.58 in addition to thirteen other signals in the aromatic region. Furthermore, the structure of the ethylenoindolophane **1** was confirmed from the appearance of the molecular ion peak at *m*/*z* 692 in the FAB-mass spectrum. Similarly the structures of the ethylenoindolophanes **1a**, **2–4** were also characterized thoroughly from the spectral and analytical data. The yields of all the synthesized compounds are given in Table 1. In general, the yields of McMurry coupling in aliphatic systems are always good, but in the case of aromatic molecules, especially in a conjugated system, the yields are low due to the π-bond conjugation, which leads to polymerization along with the cyclised dimer molecules. In our case the formation and removal of unwanted inorganic materials (Ti complex) is also responsible for low yields of the McMurry coupling (target) product.



Scheme 1 Reagents and conditions: (i) Indole (2.0 equiv.), CuI, *trans*-1,2-diaminocyclohexane, K_3PO_4, toluene, 120 °C, 24 h; **9** (82%); **9a** (85%); **10** (73%); **11** (80%); **12** (64%); (ii) $POCl_3$ (2.2 equiv.), DMF (8.8 equiv.), 0 °C to RT, 3 h; **13** (78%); **13a** (72%); **14** (56%); **15** (80%); **16** (56%); (iii) $TiCl_4$ (20 equiv.), Zn (40 equiv.), Py (2 drops), THF, reflux, 12 h, **1** (17%); **1a** (18%); **2** (15%); **3** (20%); **4** (15%).

Table 1 The yields of all of the synthesized compounds[a]

Yields (%)		
N-arylindoles	Dialdehydes	Cyclophane
9 (82%)	**13** (78%)	**1** (17%)
9a (85%)	**13a** (72%)	**1a** (18%)
10 (73%)	**14** (56%)	**2** (15%)
11 (80%)	**15** (80%)	**3** (20%)
12 (64%)	**16** (56%)	**4** (15%)

[a] Notes: yields are given for all new compounds after column purification (column chromatography on silica gel). *N*-aryl indoles: $CHCl_3$–Hexane (1 : 4, v/v) as eluent. Dialdehydes: Chloroform–methanol (99 : 1) as eluent. Cyclophanes: $CHCl_3$–Hexane (1 : 4, v/v) as eluent.

Antibacterial activity

Minimal inhibitory concentration (MIC) was taken as the lowest concentration to arrest the bacterial growth.[28] The inhibition of bacterial growth was determined visually by lack of turbidity. All the indole based ethylenophanes were effective against the tested bacterial pathogens, similar to the commercial antibiotic streptomycin. Ethylenophane **1** was very active against *Staphylococcus aureus* whereas ethylenophane **1a** was very active against *Escherichia coli* and *Proteus vulgaris* and against other tested bacterial pathogens with good inhibition zones. Ethylenophane **2** was fairly active against Gram negative bacterial pathogens and less potent for Gram positive bacteria. Ethylenophanes **3** and **4** are less active against Gram positive bacterial pathogens. The antibacterial activities of ethylenophanes **1** and **1a** are promising and hence they could be potential antimicrobial drugs. Minimum inhibitory concentrations (MIC) of the indole based ethylenophanes **1–4** are shown in Table 2.

Docking results

In order to study the biological mode of action of indole based ethylenophanes, a docking study was performed.[29] All five ethylenophanes were docked with the bacterial enzyme topoisomerase IV (PDB ID: 3FV5). We chose this target because the topoisomerases are the target of many antibiotics and other natural compounds.[30] Topoisomerase IV is an important enzyme in the process of decatenation of multiply linked daughter chromosomes during the end stage of DNA replication, thus helping in the division of cells. Topoisomerase IV is one of the type II topoisomerases in bacteria, which is responsible for unlinking or deactivating DNA following DNA replication. Topoisomerase IV helps in removal of interlinking in the two newly replicated DNA strands and thus helps in the segregation of chromosomes into daughter cells to complete cell division. When the catalytic property of this enzyme is affected, the cell division is affected, thus leading to the death of the cell. Thus we have chosen Topoisomerase IV as a target for ethylenophanes for computational study. The ethylenophanes **1–4** bind to the active pocket of the enzyme Topoisomerase IV and thus inhibit its function, which substantiates the wet lab experimental results. All five ethylenophanes show good binding energy and have hydrophobic interactions with the amino acids in the active pockets. The ethylenophane **1a** has strong hydrophobic interactions (Fig. 2) with Topoisomerase IV with high binding energy (-9.04 kcal mol^{-1}) when compared to other compounds. The three ethylenophanes *viz.* **1**, **2**, **3** show good binding energy with the enzyme and compound **4** shows the lowest binding energy and the least hydrophobic interactions. Ethylenophane **1a** has hydrophobic interactions with Pro75 (A), Ile90 (A), Thr163 (A), Met74 (A), Gly73 (A), Arg132 (A), Arg72 (A), Gly71 (A), Gly162 (A), Asp70 (A), Glu46 (A), Asp69 (A), Ser43 (A), Asn42 (A), Val67 (A) and Val165 (A). The interactions of the other compounds are diagrammatically represented using Pymol software. Even though the interactions of ethylenophanes **1**, **2**, **3** and **4** with the enzyme show the same hydrophobic interactions, ethylenophane **1a** is considered to be

Table 2 The range of minimum inhibitory concentration (MIC) values of the ethylenoindolophanes **1–4**

MIC (μg mL^{-1})				
Cyclophanes	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Proteus vulgaris*
1	7.81	15.62	15.62	15.62
1a	15.62	31.25	7.81	7.81
2	31.25	62.50	15.62	15.62
3	62.50	62.50	15.62	31.25
4	62.50	62.50	31.25	31.25
Streptomycin	3.90	3.90	3.90	7.81
Control	NI[a]	NI[a]	NI[a]	NI[a]

[a] NI – no inhibition.

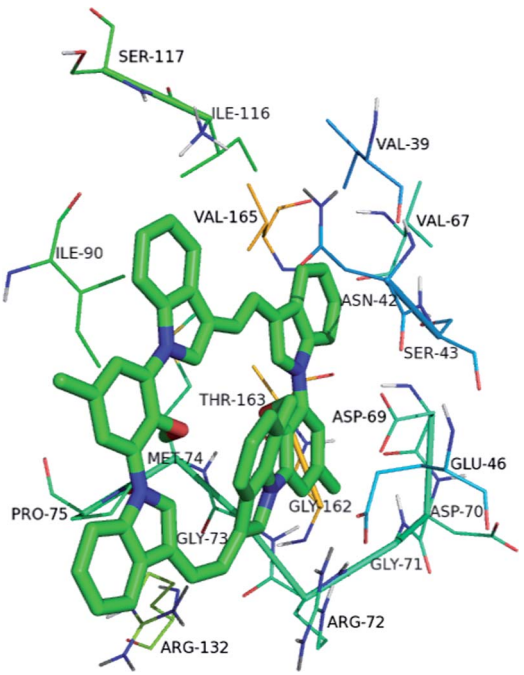


Fig. 2 Binding of ligand (Ethylenophane **1a**) with the A chain amino acids of the bacterial enzyme Topoisomerase IV (PDB ID: 3FV5).

a good lead, based on its high binding energy and high antibacterial activity, which is further supported by experimental results.

Antioxidant activity

The DPPH assay is an easy, rapid and sensitive method to screen the antioxidant activity of the given test compounds.[31] In the present study the antioxidant capacity of the ethylenophanes **1–4** were analyzed by a DPPH assay. All five ethylenophanes showed significant scavenging of the free radical generated by DPPH. The free radical scavenging property of all the ethylenophanes was tested from the lowest concentration of 10 μg mL^{-1} up to the highest concentration of 50 μg mL^{-1}. The scavenging activity increases with increasing concentration of the ethylenophanes. The free radical scavenging properties of all ethylenoindolophanes are given in Fig. 3. The ethylenophane **1a** showed excellent free radical scavenging activity at all concentrations with an IC_{50} value of 28 μg mL^{-1} and it is more effective than the other four ethylenophanes. Ascorbic acid (vitamin C) was used as a standard to measure the free radical scavenging by DPPH assay. The IC_{50} values of all compounds and the standard are given in Fig. 4.

In conclusion, we have synthesized biologically active indole based ethylenophanes **1**, **1a**, **2**, **3** and **4** from various dibromides by direct *N*-arylation methodology followed by Vilsmeier formylation and McMurry coupling for the first time. Among the ethylenophanes, ethylenophane **1a** exhibited antibacterial and antioxidant properties similar to that of standard drugs such as streptomycin for antibacterial and ascorbic acid for antioxidant activity, and hence ethylenophane **1a** may be a potential lead molecule for the synthesis of drugs with both antibacterial and antioxidant activities. Synthesis of similar types of ethylenophanes and antibacterial and antioxidant studies are underway.

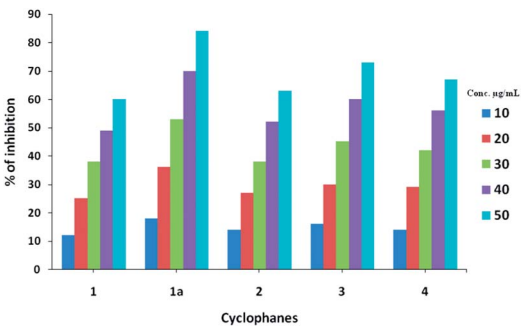


Fig. 3 *In vitro* free radical scavenging activity of the ethylenoindolophanes **1–4**.

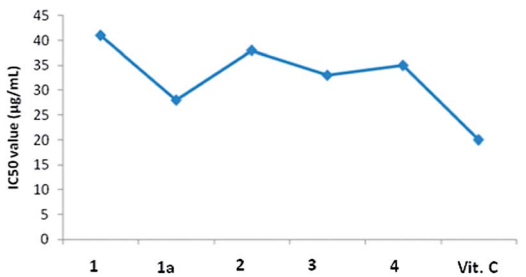


Fig. 4 IC_{50} values for the ethylenoindolophanes **1–4** and ascorbic acid (vit. C).

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28 *Antibacterial activity*: Gram positive and Gram negative bacterial cultures were used for the antibacterial study. The cultures of human pathogenic bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Proteus vulgaris* used in this study were obtained from the Culture Collections of Bio control and Microbial Metabolites Lab, Centre for Advanced Studies in Botany, University of Madras and maintained on Nutrient Agar (NA) consisting of the following (g L^{-1}): 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). The minimum inhibitory concentration was determined by a two-fold broth dilution method using Muller Hinton broth. The indole based ethylenophanes were sequentially diluted to achieve the final concentrations of 1000 μg, 500 μg, 250 μg, 12 5 μg, 62.5 μg, 31.25 μg, 15.62 μg, 7.81 μg, 3.9 μg and 1.95 μg mL^{-1}. 1 mL of bacterial culture (1×10^5 cfu mL^{-1}) was inoculated and incubated at 37 °C for 16 h. The commercial antibiotic streptomycin at different concentrations served as a positive control. The tube without ethylenophanes and tube without bacterial culture served as a negative control.

29 *Docking studies*: the molecular docking of ethylenoindolophanes was performed using Autodock version 4 with Lamarckian genetic algorithm. As a trial study to find the mode of action of the compounds, the five indole ethylenophanes were subjected to docking with the bacterial enzyme Topoisomerase IV (PDB ID: 3FV5). From the docking studies, the binding energy and interactions between ligand and protein supported the wet lab experimental results. The results are shown in 3D form using Pymol software.

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31 *Antioxidant studies*: the antioxidant capacity of the samples was determined on the basis of their free radical scavenging activity and was measured *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Stable free radical existing in DPPH is usually utilized for the detection of the radical scavenging activity in chemical analysis. The DPPH solution (0.1 mM) in methanol was prepared and was added to test tubes containing the test samples at different concentrations (10–50 μg mL^{-1}). All the tubes were incubated for 30 min and the absorbance was measured at 517 nm using a Beckman spectrophotometer. The percentage of radical scavenging ability was determined at different concentrations of compounds with D-ascorbic acid as standard. DPPH absorbs at 517 nm, and its concentration is reduced by the existence of an antioxidant. A dose response curve was plotted to determine the IC_{50} values. The following formula is used to calculate the percentage of radical scavenging.

$$\text{Radical scavenging}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

