Synthesis and spectral studies of novel biologically active macrocycles derived from O-phthalaldehyde

K. Shanker\textsuperscript{a}, P. Muralidhar Reddy\textsuperscript{a,b}, R. Rohini\textsuperscript{a}, Vadde Ravinder\textsuperscript{a*}

\textsuperscript{a}Department of Chemistry, Kakatiya University, Warangal, India
\textsuperscript{b}Department of Chemistry, National Dong Hwa University, Hualien, Taiwan

Abstract
Fifteen novel biologically active macrocycles have been synthesized by condensing orthophthalaldehyde with different primary amines and characterized by elemental analysis, IR, $^1$H, $^{13}$C NMR and mass spectral analysis. Four macrocycles were found to have potent antibacterial activity against Gram +ve and Gram –ve bacteria when compared to the two existing antibacterial drugs \textit{viz.} streptomycin and ampicillin. These compounds also tested for fungicidal activity.

Keywords: antibacterial activity, antifungal activity, macrocycles, orthophthalaldehyde

Introduction
The design and synthesis of macrocycles is one of the fascinating areas for chemists because of their biological applications \cite{1,2}. Macrocycles can be synthesized either by template approach \cite{3}, which involves the initial synthesis of macrocyclic metal complexes and the subsequent demetallation to produce macrocycles or by non-template approach \cite{4}, which involve the direct condensation of dialdehydes with diamines. Orthophthalaldehyde (OPA) serves as a good starting material for the synthesis of a large number of macrocycles \cite{5,6}. Literature survey reveals that a little bit of work was done to synthesize macrocycles from OPA by both the methods \cite{7,8}. The template approach was found to have serious limitations \cite{9} because information about the properties of the free macrocyclic ligands is not available in order to interpret and correlate the properties of the respective macrocyclic metal complexes. Hence, in the present investigations, non-template synthesis of macrocycles especially involving Schiff base condensation was chosen to overcome this problem. OPA was directly condensed with various diamines in aqueous methanol (1:1) to obtain the respective macrocycles.

Materials and methods
Analar grade samples of orthophthalaldehyde (OPA), diamines, methanol and chloroform were used throughout the investigations. The percentages of carbon, hydrogen, nitrogen were determined by using a Perkin-Elmer 240C CHN analyzer. The IR spectra were recorded in KBr pellets on Perkin Elmer-283 spectrophotometer. Brucker WH 300 (200
MHz) and Brucker WH 270 (67.93 MHz) spectrometers were used for $^1$H NMR and $^{13}$C NMR spectra. ESI and FAB MS were used to obtain mass spectra. Hot air oven (Instrument and equipment Pvt. Ltd., Mumbai), incubator (Instrument and equipment Pvt. Ltd., Mumbai), laminar airflow unit (Clas laminar technologies Pvt. Ltd. Secunderabad), autoclave (Medica instrument Mfg. Co., Mumbai) were used in the present investigations. Organisms like Bacillus subtilis (MTCC–619, IMTECH, Chandigarh), Staphylococcus aureus (MTCC–96, IMTECH, Chandigarh), Escherichia coli (MTCC–722, IMTECH, Chandigarh), Klebsiella pneumonia (MTCC–109, IMTECH, Chandigarh), Aspergillus flavus (A. flavus) (MGM Hospital, Warangal), Fusarium (MGM Hospital, Warangal), were used in the present investigations.

**General procedure for the preparation of compounds 1-15.**

In a 100 mL beaker, OPA (2.68 g, 0.02 mol.) in 30 mL of aqueous methanol (1:1) solution is taken and placed on a hot plate stirrer. To this, thiourea (1.52 g, 0.02 mol.), thiocarbohydrazide (2.12 g, 0.02 mol.), carbohydrazide (1.8 g, 0.02 mol.), thiosemicarbazide (1.82 g, 0.02 mol.), semicarbazidehydrochloride (2.23 g, 0.02 mol.), diethylenetriamine (2.06 g, 0.02 mol.), 1,2-diaminopropane (1.48 g, 0.02 mol.), 1,3-diaminopropane (1.48 g, 0.02 mol.), 1,4-diaminobutane (1.76 g, 0.02 mol.), 4-methoxy-1,2-phenylenediaminedihydro chloride (4.22 g, 0.02 mol.), 4-chloro-1,2-phenylenediamine (2.85 g, 0.02 mol.), 4,5-dichloro-1,2-phenylenediamine (3.54 g, 0.02 mol.), 4-fluoro-1,2-phenylenediamine (2.52 g, 0.02 mol.), 3,4-diaminopyridine (2.18 g, 0.02 mol.) or 2,3-diaminonaphthalene (3.16 g, 0.02 mol.) in 30 ml of aqueous methanol (1:1) solution was added drop wise. Now, an equimolar amount of sodium acetate (1.64 g, 0.02 mol.) was added to the above reaction mixture and stirred. After cooling, the precipitate formed was filtered, washed with methanol and diethyl ether in small portions, dried in vacuum and recrystallized from methanol-diethyl ether mixture. The purity of the product was checked with TLC by using chloroform and methanol mixture.

7,16-dihydrodibenzo[e,l][1,3,8,10]tetraazacyclotetradecine-7,16-dithione(DBACDT) (1). Brown color solid (2.784 g, 80%); IR: 3041, 1625, 1445, 1403, 1246 cm$^{-1}$; $^1$H NMR(200 MHz CDCl$_3$) $\delta$ 7.00-8.00 (8H, m, Ar-H), 8.40 (4H, s, CH=N); $^{13}$C NMR (67.93 MHz, CDCl$_3$) $\delta$ 134.0, 136.0, 138.2, 141.1 (12C, Ar-C), 164.4 (4C, CH=N), 193.5 (2C, C=S); Anal. found: C, 61.80; H, 3.60; N, 15.98%; Calc. for C$_{18}$H$_{12}$N$_4$S$_2$, C, 62.05; H, 3.47; N, 16.08%.

7,8,9,18,19,20-hexahydrodibenzo[g,p][1,2,4,5,10,11,13,14]octaazacyclooctadecine-8,19-dithione (HBOADT)(2). Light brown color solid (3.713 g, 88%); IR: 3325, 3060, 1497, 1439, 1248 cm$^{-1}$; $^1$H NMR (200 MHz CDCl$_3$) $\delta$ 5.80 (4H, s, NH), 7.20-7.80 (8H, m, Ar-H), 8.25 (4H, s, CH=N); $^{13}$C NMR (67.93 MHz, CDCl$_3$) $\delta$ 132.4, 133.7, 139.0 (12C, Ar-C), 164.4 (4C, CH=N), 193.5 (2C, C=S); Anal. found: C, 61.80; H, 3.60; N, 15.98%; Calc. for C$_{19}$H$_{18}$N$_8$S$_2$, C, 62.05; H, 4.29; N, 26.52%.
7,8,9,18,19,20-hexahydrodibenzo[g,p][1,2,4,5,10,11,13,14]octaazacyclooctadecine-8,19-dione (HBOADO) (3).
Pale yellow color crystalline solid (3.042 g, 78%); 1H NMR (200 MHz CDCl₃) δ 5.90 (4H, s, NH), 7.00-8.05 (8H, m, Ar-H), 8.30 (4H, s, CH=N); 13C NMR (67.93 MHz, CDCl₃) δ 131.8, 133.8, 135.0 (12C, Ar-C), 158.5 (4C, CH=N), 172.4 (2C, C=O); Anal. found: C, 58.30; H, 4.70; N, 28.90%; Calc. for C₁₉H₁₈N₈O₂, C, 58.45; H, 4.65, N, 28.70%.

7,8,17,18-tetrahydrodibenzo[f,n][1,2,4,9,11,12]hexaazacyclodocosine-8,17-dithione (TBAHD) (4).
Dark Brown color solid (2.910 g, 77%); IR: 3380, 3030, 1610, 1483, 1442, 1242 cm⁻¹; 1H NMR (200 MHz CDCl₃) δ 5.84 (2H, s, NH), 7.00-8.00 (8H, m, Ar-H), 8.10 (4H, s, CH=N); 13C NMR (67.93 MHz, CDCl₃) δ 133.4, 134.2, 135.9, 136.5, 137.6, 138.7 (12C, Ar-C), 152.4, 169.3 (4C, CH=N), 176.3 (2C, C=S); Anal. found: C, 57.50; H, 3.53; N, 22.50%; Calc. for C₁₈H₁₄N₆S₂, C, 57.12; H, 3.73; N, 22.20%.

7,8,17,18-tetrahydrodibenzo[f,n][1,2,4,9,11,12]hexaazacyclodocosine-8,17-dione (TBACD) (5).
Cream color solid (2.837 g, 82%); IR: 3322, 3020, 1666, 1605, 1425, 1403 cm⁻¹; 1H NMR (200 MHz CDCl₃) δ 5.85 (2H, s, NH), 7.10-7.90 (8H, m, Ar-H), 8.10 (4H, s, CH=N); 13C NMR (67.93 MHz, CDCl₃) δ 133.0, 134.2, 135.5, 137.3 (12C, Ar-C), 152.2, 170.0 (4C, CH=N), 156.3 (2C, C=O); Anal. found: C, 62.22; H, 3.98; N, 24.42%; Calc. for C₁₈H₁₄N₆O₂, C, 62.42; H, 4.07; N, 24.26%.

7,8,9,10,11,20,21,22,23,24-decahydrodibenzo[i,t][1,4,7,12,15,18]hexaazacyclodocosine (DHBACHD) (6).
Cream color solid (3.015 g, 75%); IR: 3320, 3060, 1626, 1430, 1410 cm⁻¹; 1H NMR (200 MHz CDCl₃) δ 2.55-2.65 (8H, t, -CH₂-NH), 3.70-3.74 (8H, t, =N-CH₂-), 5.95 (2H, s, NH), 7.25 (8H, s, Ar-H), 8.20 (4H, s, CH=N); 13C NMR (67.93 MHz, CDCl₃) δ 45.3 (4C, -CH₂-NH), 60.0 (4C, =N-CH₂-), 131.9, 133.5, 135.9, 136.0 (12C, Ar-C), 163.4 (4C, CH=N); Anal. found: C, 71.52; H, 7.60; N, 20.68%; Calc. for C₂₄H₃₀N₆, C, 71.60; H, 7.50; N, 20.80%.

7,17-dimethyl-7,8,17,18-tetrahydrodibenzo[i,t][1,4,7,12,15,18]hexaazacyclodocosine (MTDAHD) (7).
Yellow color fine powder (2.680 g, 78%); IR: 3016, 1618, 1479, 1341 cm⁻¹; 1H NMR (200 MHz CDCl₃) δ 1.38-1.40 (6H, d, -CH₃), 2.96-3.00 (4H, d, -CH₂), 4.20-4.35 (2H, m, -CH-), 7.20-7.75 (8H, m, Ar-H), 8.09 (4H, s, CH=N); 13C NMR (67.93 MHz, CDCl₃) δ 20.2 (2C, -CH₃), 56.9 (2C, -CH₂), 60.0 (2C, -CH₂), 131.2, 133.4, 135.4, 136.0, 138.7, 139.0 (12C, Ar-C), 162.9 (4C, CH=N); Anal. found: C, 76.60; H, 6.94; N, 16.40%; Calc. for C₂₂H₂₄N₆, C, 76.71; H, 7.02; N, 16.27%.

7,8,9,10,11,20,21,22,23,24-decahydrodibenzo[i,t][1,4,7,12,15,18]hexaazacyclodocosine (HDBACHD) (8).
Pale yellow crystalline solid (2.614 g, 76%); IR: 3040, 1615, 1430, 1345 cm⁻¹; 1H NMR (200 MHz CDCl₃) δ 2.42-2.50 (4H, m, -CH₂-), 3.90-3.98 (8H, t, N-CH₂-), 7.20-7.60 (8H,
m, Ar=H), 8.10 (4H, s, CH=N); \(^{13}\)C NMR (67.93 MHz, CDCl\(_3\)) \(\delta\) 24.2 (2C, -CH\(_2\)-), 54.8 (4C, N-CH\(_2\)), 132.2, 133.3, 135.0, 136.5 (12C, Ar-C), 160.0 (4C, CH=N); Anal. found: C, 76.80; H, 7.15; N, 16.02%; Calc. for C\(_{22}\)H\(_{24}\)N\(_4\), C, 76.71; H, 7.02; N, 16.27%.

7,8,9,10,19,20,21,22-octahydrodibenzo[c,m][1,6,11,16]tetraazacycloicosine (OBACI) (9). Cream color fine powder (3.05 g, 82%); IR: 3015, 1630, 1420, 1350 cm\(^{-1}\); \(^1\)H NMR (200 MHz CDCl\(_3\)) \(\delta\) 2.15-2.30 (8H, m, -CH\(_2\)-CH\(_2\)-), 3.50-3.60 (8H, m, -CH\(_2\)-N), 7.20-7.50 (8H, m, Ar-H), 8.19 (4H, s, CH=N); \(^{13}\)C NMR (67.93 MHz, CDCl\(_3\)) \(\delta\) 26.7 (4C, -CH\(_2\)-CH\(_2\)-), 58.2 (4C, -CH\(_2\)-N), 131.7, 133.3, 135.0, 136.8 (12C, Ar-C), 160.7 (4C, CH=N); Anal. found: C, 77.50; H, 7.60; N, 14.92%; Calc. for C\(_{24}\)H\(_{28}\)N\(_4\), C, 77.38; H, 7.58; N, 15.04%.

2,15-dimethoxytetrabenzo[b,f,j,n][1,4,9,12]tetraazacyclohexadecine (DMBACHD) (10). Light yellow solid (3.776 g, 80%); IR: 3020, 1616, 1416, 1380, 1160 cm\(^{-1}\); \(^1\)H NMR (200 MHz CDCl\(_3\)) \(\delta\) 3.74 (6H, s, -OCH\(_3\)), 6.80-7.65 (14H, m, Ar-H), 8.09 (4H, s, CH=N); \(^{13}\)C NMR (67.93 MHz, CDCl\(_3\)) \(\delta\) 56.1 (2C, -OCH\(_3\)), 110.5, 111.2, 122.6, 131.4, 133.5, 143.2, 148.0 (24C, Ar-C), 166.9 (4C, CH=N); Anal. found: C, 76.02; H, 5.20; N, 12.02%; Calc. for C\(_{30}\)H\(_{24}\)N\(_4\)O\(_2\), C, 76.25; H, 5.12; N, 11.86%.

2,15-dichlorotetrabenzo[b,f,j,n][1,4,9,12]tetraazacyclohexadecine (DCBACHD) (11). Yellow solid (3.799 g, 79%); IR: 3020, 1625, 1425, 1330, 762 cm\(^{-1}\); \(^1\)H NMR (200 MHz CDCl\(_3\)) \(\delta\) 7.20-7.60 (14H, m, Ar-H), 8.04 (4H, s, CH=N); \(^{13}\)C NMR (67.93 MHz, CDCl\(_3\)) \(\delta\) 124.3, 126.0, 128.1, 131.4, 132.9, 133.5, 137.0, 141.8, 142.5 (24C, Ar-C), 168.0 (4C, CH=N); Anal. found: C, 70.02; H, 3.60; N, 11.55%; Calc. for C\(_{28}\)H\(_{18}\)N\(_4\)Cl\(_2\), C, 69.86; H, 3.77; N, 11.64%.

2,3,14,15-tetrachlorotetrabenzo[b,f,j,n][1,4,9,12]tetraazacyclohexadecine (TABACHD) (12). Dark yellow solid (4.235 g, 77%); IR: 3015, 1620, 1480, 1440, 774 cm\(^{-1}\); \(^1\)H NMR (200 MHz CDCl\(_3\)) \(\delta\) 7.30-7.64 (12H, m, Ar-H), 8.08 (4H, s, CH=N); \(^{13}\)C NMR (67.93 MHz, CDCl\(_3\)) \(\delta\) 126.6, 131.4, 133.5, 136.8, 142.5 (24C, Ar-C), 166.2 (4C, CH=N); Anal. found: C, 61.02; H, 3.05; N, 10.25%; Calc. for C\(_{28}\)H\(_{16}\)N\(_4\)Cl\(_4\), C, 61.12; H, 2.93; N, 10.18%.

2,15-difluorotetrabenzo[b,f,j,n][1,4,9,12]tetraazacyclohexadecine (DFBACHD) (13). Pale yellow solid (3.673 g, 82%); IR: 3012, 1622, 1475, 1420, 945 cm\(^{-1}\); \(^1\)H NMR (200 MHz CDCl\(_3\)) \(\delta\) 6.80-7.50 (14H, m, Ar-H), 8.02 (4H, s, CH=N); \(^{13}\)C NMR (67.93 MHz, CDCl\(_3\)) \(\delta\) 110.9, 111.6, 117.5, 122.3, 123.0, 133.5, 136.0, 142.2 (22C, Ar-C), 1630 (2C, Ar-C-F), 167.5 (4C, CH=N); Anal. found: C, 75.10; H, 3.92; N, 12.60%; Calc. for C\(_{28}\)H\(_{18}\)N\(_4\)F\(_2\), C, 74.99; H, 4.05; N, 12.49%.

dibenzo[f,n]dipyrido[3,4-b:4,3-j][1,4,9,12]tetraazacyclohexadecine (DDPACHD) (14). Light pink color solid (3.519 g, 85%); IR: 3020, 1628, 1595, 1435, 1405 cm\(^{-1}\); \(^1\)H NMR (200 MHz CDCl\(_3\)) \(\delta\) 7.10-7.85 (12H, m, Ar-H), 8.10 (2H, s, Ar-CH=N), 8.50 (4H, s, CH=N); \(^{13}\)C NMR (67.93 MHz, CDCl\(_3\)) \(\delta\) 118.0, 131.2, 133.1, 138.5, 142.2, 146.3, 147.0 (20C, Ar-C), 156.0 (2C, Ar-CH=N), 170.5 (4C, CH=N); Anal. found: C, 75.50; H, 4.20; N, 20.36%; Calc. for C\(_{28}\)H\(_{18}\)N\(_6\), C, 75.35; H, 4.38; N, 20.28%. 

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dibenzo[f,n]dinaphtho[2,3-b:2,3-j] tetraazacyclohexadecine (DBNACHD)(15). Light pink color solid (4.096 g, 80%); IR: 3020, 1612, 1420, 1407 cm⁻¹; ¹H NMR (200 MHz CDCl₃)  δ 7.10-7.80 (20H, m, Ar-H), 8.04 (4H, s, CH=N); ¹³C NMR (67.93 MHz, CDCl₃)  δ 117.5, 125.0, 128.0, 131.5, 133.6, 137.8, 139.0 (3 2C, Ar-C), 166.2 (4C, CH=N); Anal. found: C, 84.40; H, 4.55; N, 11.02%; Calc. for C₃₆H₂₄N₄, C, 84.35; H, 4.72; N, 10.93%.

**Antimicrobial testing by agar diffusion**

Antimicrobial testing was done by cup plate method [10]. 27 ml of molten agar was added in to sterile Petri dishes and allowed to solidify for 1hr. Then 50 ml of the 24 hrs culture of a test organism was spread evenly on to the agar plate with the sterile cotton swab. Six mm wide bores were made on the agar using a borer. The solutions of the macrocyclic compounds were added in to each of the bores using a sterile tip with micropipette. A similar plate was prepared by replacing macrocycle by Streptomycin sulphate. This was taken as a standard against bacteria. These dishes were then incubated at 37°C for 24 hr. The zones of growth inhibition were found. The activities of compounds were interpreted either active or inactive. The minimum inhibitory concentration required was also found when a series of dilutions were tested.

**Determination of minimum inhibitory concentration (MIC)**

The minimum inhibitory concentration [11] was determined by liquid dilution method. Stock solutions of macrocyclic compounds with 2.5 µg/ml, 5 µg/ml, 10µg/ml, 20 µg/ml, 50µg/ml and 100µg/ml concentrations were prepared with appropriate solvent. The solutions of standard drugs like Streptomycin and Ampicillin were also prepared in the same concentrations. Inoculums of the overnight culture were prepared. To a series of tubes containing 1 ml each of macrocyclic compound solution with different concentrations and 0.2 ml of the inoculum was added. Further 3.8 ml of the sterile water was added to each of the test tubes. These test tubes were incubated for 24 hrs and observed for the presence of turbidity. The absorbance of the suspension of the inoculum was detected using a spectrophotometer at 550 nm. This method was repeated by changing macrocyclic compounds with drugs like Streptomycin and Ampicillin for comparison.

**Determination of antifungal activity**

Macrocyclic compounds were tested for their in vitro growth inhibitory activity against the pathogenic fungus, namely A. flavus and Fusarium species cultured on sabour dextrose agar medium prepared by taking 11 ml of distilled water in a conical flask followed by the addition of following ingredients: mycological peptone, 10 g; dextrose, 30 g; agar, 12 g; and the pH of the solution was adjusted to 5.7 boiling was continued until complete dissolution. After that, the solution was sterilized by autoclaving at 15 lb pressure (120 °C for 20 min) by diffusion method [12-14] and incubated at 28 °C for 3 days. Several test solutions of different concentration (microgram per liter) were prepared in water-methanol solution. The percentage inhibition of fungal growth was determined on the growth in test plates compared to that of respective control plates, given by the Vincent equation [15]. Percentage inhibition = 100(C-T )/C, Where C is the diameter of
fungal growth on the control plate, and \( T \) is the diameter of fungal growth on the test plate.

**Results and Discussion**

In the present investigations, fifteen macrocyclic compounds were synthesized by direct condensation of o-phthalaldehyde with the corresponding diamines in 2:2 ratios (Scheme-1). All these compounds were characterized by elemental, IR, \(^1\)H, \(^{13}\)C NMR and mass spectral analysis.

IR spectra of macrocycles, a strong intensity band are appeared in the range of 1630-1600 cm\(^{-1}\) which is attributed to \( \nu_{C=N} \) provides a strong evidence for the presence of cyclic product [16]. The IR spectra of compounds 2, 3, 4, 5 and 6 show a broad band in the range of 3380-3315 cm\(^{-1}\) corresponding to \( \nu_{NH} \) frequency [17]. The compounds 1, 2 & 4 show a signal around 1245 cm\(^{-1}\) and the compounds 3 & 5 show a band around 1665 cm\(^{-1}\) corresponding to \( \nu_{C=S} \) and \( \nu_{C=O} \) respectively [18,19]. Similarly, for compound 14, a characteristic absorption band at 1595 cm\(^{-1}\) corresponds to \( \nu_{C=N} \) (pyridine) was observed [20]. Aromatic ring stretching frequencies were observed for all the compounds in the range of 1497-1330 cm\(^{-1}\) and wagging frequencies were observed in the range of 3060-3012 cm\(^{-1}\) [9]. The IR spectrum of compound 7,16-dihydrodibenz[e,l][1,3,8,10]tetraazacyclotetradecine-7,16-dithione (1) is presented as figure-1 in supplementary material.

In the \(^1\)H NMR spectra, the integral intensities of each signal were found to agree with the number of different types of protons present. In all the spectra, a singlet was appeared from CH=N protons (4H) in the range of 8.02-8.50 \( \delta \) suggest the formation of a macrocyclic framework by the condensation of OPA with primary amines [21]. This fact was also supported by the disappearance of peaks around 9.98 \( \delta \) corresponding to aldehydic protons of OPA [9]. However, in the spectra of compounds 2, 3, 4, 5 & 6, a broad signal in the range of 5.80-5.95 \( \delta \) was found indicating the presence of NH proton [22]. In compound 6, two triplets were observed in the ranges of 2.55-2.65 \( \delta \) corresponding to methylenic protons adjacent to different nitrogen atoms. In compound 8, multiplet and triplet were observed in the ranges 2.42-2.50 \( \delta \) and 3.90-3.98\( \delta \), respectively, corresponding to -CH\(_2\)- and N-CH\(_2\)- groups. Where as, in compound 9, two multiplets in the ranges of 2.15-2.30 \( \delta \) and 3.50-3.60 \( \delta \) were observed corresponding to methylenic protons. Compound10, showed a singlet at 3.74 due to the methoxy protons [23]. Multiplets observed in the ranges of 6.80-8.05 \( \delta \) have been assigned to the aromatic protons [9]. The \(^1\)H NMR spectrum of 7,16-dihydrodibenzo[e,l][1,3,8,10]tetraazacyclotetradecine-7,16-dithione (1) is presented as figure-2 in supplementary material. \(^{13}\)C NMR spectra of all the compounds contain signals in the range of 152.2-170.0 \( \delta \) indicating the presence of carbon which is doubly bonded to nitrogen [24]. The spectra of compounds 1, 2 & 4 contain signals in the range of 174.5-193.5 \( \delta \) corresponding to C=S carbons [25]. The spectra of compounds 3 and 5 contain signals in the range of 156.3-172.0 \( \delta \) indicating the presence of carbonyl carbon [26]. Similarly, the spectra of compounds 6-10, having methylenic carbons contain...
signals in the range of 20.2-60.0$\delta$. The spectra of compound 14 contain a signal at 156.0$\delta$ indicating the presence of carbon adjacent to nitrogen in pyridine ring [24].

\[
\begin{align*}
  \text{O} & \quad \text{N} \\
  \text{S} & \quad \text{X} \\
  \text{N} & \quad \text{X} \\
  \text{Cl} & \quad \text{Cl} \\
  \text{OMe} & \quad \text{X} \\
  \text{NH} & \quad \text{NH} \\
  \text{N} & \quad \text{O} \\
  \text{H} & \quad \text{S} \\
  \text{H} & \quad \text{H}
\end{align*}
\]

where $-\text{X-} =$

\[
\begin{align*}
  \text{S} & \quad \text{N} \\
  \text{HN} & \quad \text{NH} \\
  \text{HN} & \quad \text{NH} \\
  \text{S} & \quad \text{N} \\
  \text{O} & \quad \text{N} \\
  \text{NH} & \quad \text{NH} \\
  \text{O} & \quad \text{N} \\
  \text{N} & \quad \text{H}
\end{align*}
\]

for DBACDT (1)

for HBOADT (2)

for HBOADO (3)

for TBAHD (4)

for TBACD (5)

for DHBACHD (6)

for MTDAHD (7)

for HDBACHD (8)

for ODBACI (9)

for DMBACHD (10)

for DCBACHD (11)

for TCBACHD (12)

for DFBACHD (13)

for DDPACHD (14)

for DBNACHD (15)

**Scheme 1.** Synthetic route of macrocyclic compounds.
The aryl carbons were resonated in the range of 110.5-148.0 $\delta$ The $^{13}$C NMR spectrum of 7,16-dihydrodibenzo[e,l][1,3,8,10]-tetraazacyclotetradecine-7,16-dithione (1) is presented as figure-3 in supplementary material.

The mass spectra of compounds viz. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 showed the molecular ion peaks at m/z (M$^+$) 348(18%), 422(9%), 390(12%), 378(5%), 346(4%), 402(5%), 344(10%), 344(6%), 372(12%), 472(5%), 481(8%), 550(4%), 448(14%), 414(10%) and 512(9%) respectively. This data is in good agreement with the respective molecular formulae. However, a complete fragmentation of 7,16-dihydrodibenzo[e,l][1,3,8,10]tetraazacyclotetradecine-7,16-dithione(1) is only discussed in detail for brevity.

The fast atom bombardment (FAB) mass spectrum of 1 shows a parent peak at m/z 348 (18%) which supports the formation of condensed product formed from o-phthalaldehyde and thiourea in the 2:2 ratios, corresponding to the molecular formula C$_{18}$H$_{12}$N$_4$S$_2$. This contains peaks corresponding to major fragments at m/z values of 321 (62.5%), 290 (75.2%), 278 (26.4%), 218 (100%), 173 (42%), 160 (22.5%) and 103 (55.4%). The mass fragmentation pattern of 7, 16-dihydrodibenzo[e,l][1,3,8,10]tetraazacyclotetradecine-7,16-dithione (1) as assigned on the basis of FAB mass spectrum (Figure-4) is presented as scheme-1 in supplementary material.

On the basis of elemental analysis, IR, $^1$H NMR and $^{13}$C NMR and mass spectral data macrocyclic frame work has been assigned for the newly synthesized compounds.

**Antibacterial Activity**

Antibacterial activities of the present macrocycles were studied along with two existing antibacterial drugs viz. streptomycin and ampicillin. Preliminary screening for all the macrocycles was performed at fixed concentrations of 1000 µg/ml (Table 1). All the compounds were found to be acting on two types each of gram+ve and gram–ve bacteria. Out of fifteen macrocycles, only four compounds i.e. 1, 2, 4 and 14 were found to be very effective based on the obtained values of relative zone of inhibition. In addition, the above four macrocycles were found to be effective at different dilutions based on the activity. The minimum inhibitory concentration of these macrocycles was also verified by the liquid dilution method in which the effectiveness was observed at lower concentrations (Table 2). Rank orders of the relative effectiveness of these four compounds against gram+ve and gram–ve bacteria were obtained in which 14 was found to be at the top in rank order. The activity of these active four macrocycles against gram+ve and gram–ve bacteria were compared with the activity of existing antibacterial drugs like Streptomycin and Ampicillin and these macrocycles were found be very active than these two drug molecules.
**Table 1.** Zone of inhibition of macrocyclic compounds against four different bacteria

<table>
<thead>
<tr>
<th>Compound (1000 µg/ml)</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTCC- 619</td>
</tr>
<tr>
<td>STD 1</td>
<td>10</td>
</tr>
<tr>
<td>STD 2</td>
<td>11</td>
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<tr>
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<tr>
<td>14</td>
<td>37</td>
</tr>
<tr>
<td>15</td>
<td>11</td>
</tr>
</tbody>
</table>


**Table 2.** MIC of the macrocyclic compounds and existing antibiotics

<table>
<thead>
<tr>
<th>Entry</th>
<th>Bacteria</th>
<th>Range of concentration (µg/ml)</th>
<th>Absorbance of Suspension</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Comp. 1</td>
<td>Comp. 2</td>
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<tr>
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<tr>
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<td>MTCC-96</td>
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<tr>
<td>4</td>
<td>MTCC-109</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Antifungal Activity
All the macrocycles were also tested in vitro to evaluate their fungicidal activity against Aspergillus flavus (A. flavus) and Fusarium species. The activity is tested in two different concentrations (500µg/ml & 1000µg/ml) against both fungi. The compounds 1, 2, 4 and 14 were found to increased activity as compared to other macrocyclic compounds. Though there is sufficient increase in the fungicidal activity of macrocycles, but it could not reach the effectiveness of the conventional fungicide Amphotericin and Bavistin. The zone of inhibition of macrocyclic compounds and drug molecules are presented in Fig. 1.

Figure 1. Comparison of zone of inhibition of macrocyclic compounds and existing drug molecules against two different fungi.

Conclusions
Fifteen macrocyclic compounds were synthesized by non-template method which involves the direct condensation of o-phthalaldehyde with different primary diamines. Macro cyclic frame work has been assigned for these compounds on the basis of elemental analysis, IR, $^1$H NMR, $^{13}$C NMR and mass spectral data. All the compounds showed good antibacterial activity against gram+ve and gram–ve bacteria and in particular, four of these compounds showed more antibacterial activity than the two existing antibacterial drugs.

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