European Journal of Medicinal Chemistry 44 (2009) 2621–2625

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Physicochemical and biological characterization of novel macrocycles derived from *o*-phthalaldehyde

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ARTICLE INFO

Article history:
Received 31 March 2008
Received in revised form 6 September 2008
Accepted 11 September 2008
Available online 7 October 2008

Keywords:
Physicochemical
o-Phthalaldehyde
Macrocyclic compounds
Antimicrobial activity

ABSTRACT

A series of novel macrocyclic compounds were synthesized by the condensation of *o*-phthalaldehyde with aromatic amino alcohols followed by treatment with 1,2-dibromoethane or 1,3-dibromopropane in non-template method. The structural features of the isolated macrocycles have been determined from the microanalytical, IR, ^{1}H, ^{13}C NMR and mass spectral studies. Antimicrobial activities of these macrocyclic compounds were tested against the Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) bacteria and found to exhibit potential antibacterial activity. The macrocycles were also tested *in vitro* to evaluate their activity against fungi, namely, *Aspergillus flavus* (*A. flavus*) and *Fusarium* species.

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1. Introduction

Macrocyclic compounds possessing heteroatoms are valuable compounds with broad spectrum of pharmacological activities [1–4] apart from their potential application in the field of molecular catalysis. More importantly, macrocyclic Schiff bases and the relevant transition metal complexes are of great interest in coordination chemistry, although this subject has been extensively studied [5–7]. These compounds exhibit biological activity as antibiotics, antiviral, antitumour, antifungal, antibacterials and anticancer agents because of their specific structures [8–11]. Interest in the chemistry and biological activity of tetradentate macrocyclic compounds and its complexes has increased in recent years, mainly due to their relevance to the bioorganic and bioinorganic chemistry of metal complexes [12–16]. Particular attention has been devoted to their correlation with the active sites of metalloenzymes and metalloproteins and to mimicing the activity of such biomolecules [17–20].

Macrocyclic Schiff bases provide a method of establishing multiple N_2O_2 donor sites capable of forming multimetallic complexes. Template synthesis with metal ions is the usually employed route to get macrocyclic Schiff bases. However, template synthesis has two substantial disadvantages. Firstly, more complete the template condensation, stronger the metal ion bound in the macrocyclic cavity. Hence it is difficult to isolate the free ligand by demetallation from the macrocyclic complex [2,21]. Secondly, template synthesis from dicarbonyl compounds and diamines usually affords symmetric macrocyclic complexes and thus other starting building blocks have to be used to obtain nonsymmetric macrocyclic Schiff bases [22–25]. To the best of our knowledge, no work has been done on the condensation of *o*-phthalaldehyde with aromatic amino alcohols by non-template methods. Hence, we report here the non-template synthesis of new macrocyclic compounds with characterization, antibacterial and antifungal activity. These compounds behave as tetradentate ligands by donating four lone pairs of electrons two each from nitrogen atoms and other two each from oxygen atoms.

2. Results and discussion

2.1. Chemistry

The macrocyclic compounds were derived from the condensation reaction of *o*-phthalaldehyde with aromatic amino alcohols followed by ring closure with aliphatic dihaloalkane compounds. The condensation reaction occurred efficiently in a water–methanol (1:1) suspension medium [26] without using any acid catalyst and the products were isolated by simple filtration.

The intermediates required for the synthesis of the new macrocycles, the diimine–dihydroxy compounds, were prepared by condensation reaction between 1 mol of *o*-phthaladehyde **1** and 2 mol of aromatic aminoalcohol viz. *o*-aminophenol **2**, 3-hydroxy-2-aminopyridine **3**, 3-amino-2-naphthol **4**, 2-amino-*m*-cresol **5** or

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doi:10.1016/j.ejmech.2008.09.035

2-amino-*p*-cresol **6**. The reactions afforded diimines **7**–**11**, respectively. Subsequently, [1 + 1] reaction of the diimines, **7**–**11** with 1,2-dibromoethane (**12**) or 1,3-dibromopropane (**13**) in the presence of sodium hydroxide in aqueous methanol resulted in the isolation of pure macrocycles **14**–**23** in high yields (Scheme 1).

2.1.1. Infrared spectral analysis

The appearance of a strong intensity band in the IR spectra of macrocyclic ligands (**14**–**23**), in the range of 1622–1608 cm^{-1} attributable to $\nu_{C=N}$ provides a strong evidence for the condensation [27]. Similarly, the disappearance of bands corresponding to ν_{O-H} (aromatic) and ν_{C-Br} in the range of 3512–3430 and 700–600 cm^{-1} [28] suggests the cyclization by the reaction between phenolic groups and alkyl dihalide. This fact was further supported by the appearance of bands in the range of 1208–1140 cm^{-1} corresponding to ν_{C-O-C} modes [29]. Aromatic ring stretching frequencies were observed for all the ligands around 1400 cm^{-1} region and wagging frequencies were observed around 3050 cm^{-1} region [23].

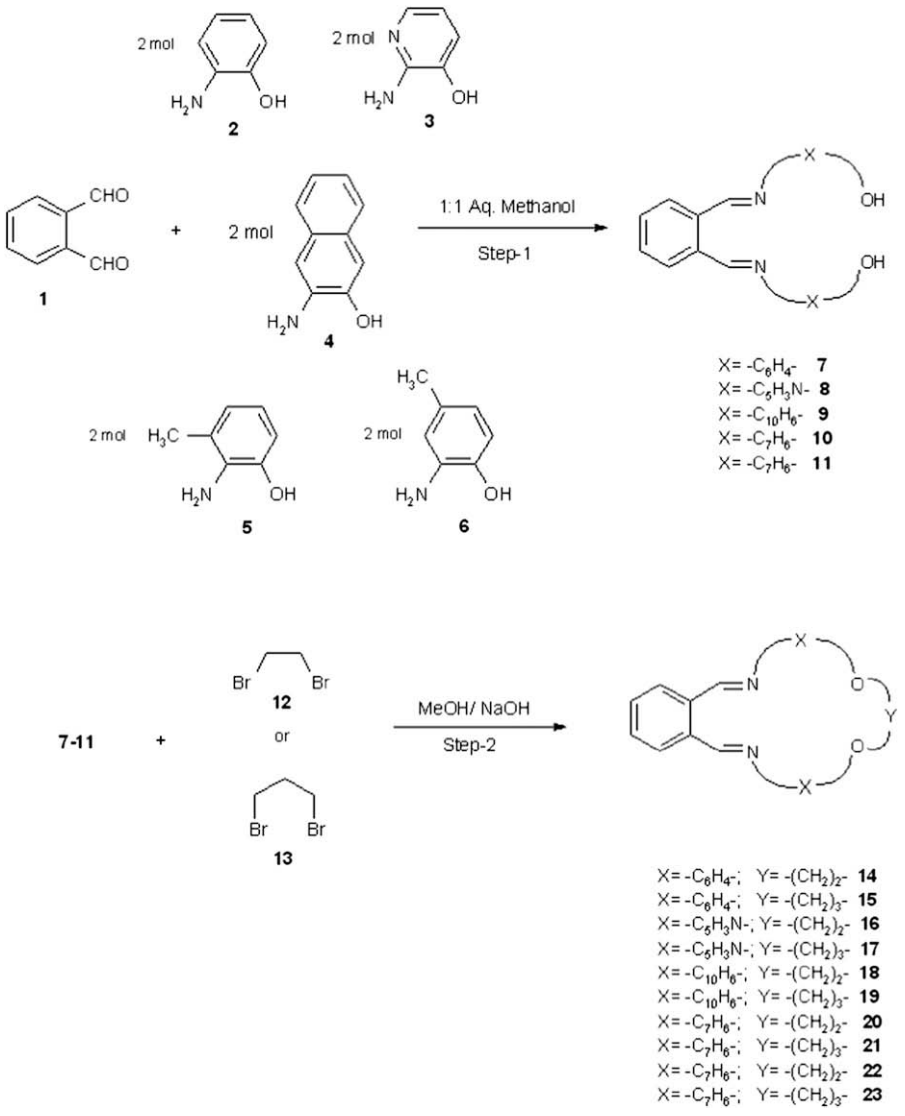
2.1.2. ^{1}H and ^{13}C NMR spectral analysis

In the ^{1}H NMR spectra of macrocyclic ligands (**14**–**23**), the integral intensities of each signal were found to agree with the number of different types of protons present. A signal arising from CH=N protons appeared in the range of 8.41–8.80 ppm suggesting the condensation of OPA with primary amines [30]. The formation of Ar–O–C linkage was further supported by the presence of signals in the range of 3.90–4.37 ppm corresponding to methylenic protons adjacent to phenolic oxygen [31,32]. Multiplets observed in the range of 6.00–7.90 ppm have been assigned to the aromatic protons [23].

^{13}C NMR spectra of all the ligands contain signals in the range of 165.0–170.2 ppm confirming the presence of carbon, which is doubly bonded to nitrogen [33]. The signals in the range of 60.0–67.4 ppm due to methylenic carbon adjacent to oxygen atoms indicate the presence of O–CH_2 linkage [34]. The aryl carbons are resonated in the range of 107.6–153.0 ppm [30].

2.1.3. FAB mass analysis

All the macrocyclic ligands displayed a single peak in ESI–MS suggesting the purity of the macrocycles. The FAB mass spectrum of compound **14** shows a parent peak at *m*/*z* (M^{+}) 342 (16.7%) corresponding to the molecular formula $C_{22}H_{18}N_2O_2$. The mass spectrum shows major fragments at *m*/*z* values of 315 (100%), 312 (74.5%), 282 (62.6%), 249 (52.1%), 222 (48.2%), 212 (31.2%), 179 (23.4%) and 43 (10.4%).



Scheme 1. Synthetic route of macrocyclic compounds.

2.2. Antimicrobial activity

Antibacterial activities of the macrocyclic compounds (**14–23**) were studied along with existing antibacterial drugs viz. Streptomycin, Ampicillin and Rifampicin. Preliminary screening for ten 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-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) bacteria. Out of ten macrocycles, only two compounds **16**, **17** were found to be very effective based on the obtained values of relative zone of inhibition. In addition, the above two macrocycles were found to be effective at different dilutions based on the activity. The minimum inhibitory concentration [35,36] of these macrocycles was also verified by the liquid dilution method in which the effectiveness was observed at lower concentrations. The activity of these two macrocycles against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) bacteria were compared with the activity of existing antibacterial drugs like Streptomycin, Ampicillin and Rifampicin and these macrocycles were found to be very active than the Streptomycin and Ampicillin (Table 2). The compounds **16**, **17** could show very good efficacy on clinical resistant strains.

2.3. Antifungal activity

The macrocyclic compounds were also tested in vitro to evaluate their activity against fungi, namely, *Aspergillus flavus* (*A. flavus*) and *Fusarium* species. Though there is sufficient increase in the fungicidal activity of macrocyclic compounds, it could not reach the effectiveness of the conventional fungicide Amphotericin and Bavistin. The general trend of growth of inhibition against the fungui were found to lie in the order: **16** > **17** > **20** > **18** = **22** > **19** = **21** > **14** > **23** > **15** for *A. flavus* (500 μg/ml), **16** > **17** > **20** > **18** = **21** = **22** > **19** = **23** > **14** > **15** for *A. flavus* (1000 μg/ml), **17** > **16** > **20** > **22** > **14** = **19** = **21** > **23** > **18** > **15** for *Fusarium* (500 μg/ml) **17** > **16** > **20** > **19** = **22** > **14** > **21** > **23** > **18** > **15** for *Fusarium* (1000 μg/ml). The results are presented in Fig. 1.

3. Experimental

o-Phthalaldehyde and substituted aromatic amino alcohols were obtained from Aldrich, USA and all other compounds are analytical grade products from Merck. The solvents were distilled and stored over molecular sieves. Purity of the compounds was checked by TLC using Merck 60F254 silica gel plates. The percentages of carbon, hydrogen, nitrogen in macrocyclic metal compounds were determined using a Perkin–Elmer CHN analyzer at 240C. The IR spectra were recorded in KBr pellets on a 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 measurements. 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.

Table 1
Zone of inhibition of macrocyclic compounds against four different bacteria.

Entry	Empirical formula (1000 μg/ml)	Zone of inhibition (mm)			
		MTCC-619	MTCC-96	MTCC-722	MTCC-109
STD 1	Streptomycin	10	12	6	6
STD 2	Ampicillin	11	13	8	7
STD 3	Rifampicin	51	49	48	45
14	$C_{22}H_{18}N_2O_2$	11	12	10	12
15	$C_{23}H_{20}N_2O_2$	10	11	11	12
16	$C_{20}H_{16}N_4O_2$	38	35	40	32
17	$C_{21}H_{18}N_4O_2$	35	30	32	29
18	$C_{30}H_{22}N_2O_2$	15	15	14	14
19	$C_{31}H_{24}N_2O_2$	13	14	13	11
20	$C_{24}H_{22}N_2O_2$	12	14	11	12
21	$C_{25}H_{24}N_2O_2$	13	15	12	11
22	$C_{24}H_{22}N_2O_2$	14	12	13	10
23	$C_{25}H_{24}N_2O_2$	13	14	11	12

3.1. Typical procedure for the preparation of macrocyclic compounds (*14–23*)

In a typical procedure, to a suspension of *ortho*-phthalaldehyde (1.34 g, 1 mmol) in 15 ml of 1:1 aqueous methanol solution was added to a 15 ml solution of *o*-aminophenol (2.18 g, 2 mmol), 2-amino-3-hydroxy pyridine (2.20 g, 2 mmol), 3-amino-2-naphthol (3.18 g, 2 mmol), 2-amino-*m*-cresol (2.46 g, 2 mmol), 2-amino-*p*-cresol (2.46 g, 2 mmol), with constant stirring at 60 °C. The reaction mixture was continuously stirred for 30 min to separate the solid from reaction mixture and cooled. The solid was filtered and washed with diethyl ether and recrystallized from methanol. The intermediate compounds **7–11** (1 equiv.), in methanol solution (30 ml) were added to an aqueous sodium hydroxide (1 equiv.) solution. This solution was heated and 1,2-dibromoethane or 1,3-dibromopropane (1 equiv.) in methanol (20 ml) was added to it. The solution was refluxed under nitrogen atmosphere for 4 h and then cooled to 0 °C. The crystals obtained were filtered and washed with methanol and water. The corresponding products were recrystallized from methanol and found to be TLC-pure in chloroform and methanol mixture. The yield, analytical data and the spectral data are given below.

The advantages of synthetic procedure are: (1) the process is a simple two-step reaction involving inexpensive starting materials; (2) the process is shorter; (3) using water–methanol (1:1) as solvent allows the formation of imines without the need for catalysis and (4) yields are high and reactions are fast, and products can be isolated by filtration.

3.1.1. 6,7-Dihydrotribenzo[e,i,m][1,4,7,12]dioxadiazacyclotetradecine (*14*)

Yield 81%; mp 181; IR 1622, 1141, 3040w, 1498, 1420, 1350 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.20 (4H, s, O–CH_2), 6.60–7.80 (12H, m, Ar-H), 8.50 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 64.0 (2C, O–CH_2–CH_2–O), 110.0, 128.0, 129.0, 133.0, 138.0, 153.0 (18C, Ar-C), 165.0 (2C, CH=N); mass spectrum, *m*/*z* 342 (6% M^+). Anal. found: C, 77.10; H, 5.40; N, 8.20%. Calcd for $C_{22}H_{18}N_2O_2$: C, 77.17; H, 5.30; N, 8.18%.

3.1.2. 13,14-Dihydro-12H-tribenzo[b,f,j][1,12,4,9]dioxadiazacyclopentadecine (*15*)

Yield 74%; mp 178; IR 1620, 1171, 3040w, 1490, 1420, 1350 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.30–2.50 (2H, m, C–CH_2–C), 3.90 (4H, t, O–CH_2), 6.80–7.80 (12H, m, Ar-H), 8.50 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 30.0 (1C, C–CH_2–C), 67.0 (2C, O–CH_2), 127.0, 128.0, 129.0, 139.0, 152.0 (18C, Ar-C), 166.0 (2C, CH=N); mass spectrum, *m*/*z* 356(8% M^+). Anal. found: C, 77.05; H, 5.33; N, 7.96%. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86%.

3.1.3. 6,7-Dihydrobenzo[i]dipyrido[3,2-e:2,3-m][1,4,7,12]dioxadiazacyclotetradecine (*16*)

Yield 78%; mp 193; IR 1625, 1584, 1206, 3042w, 1445, 1403.8, 1391 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.10 (4H, s, O–CH_2),

Table 2
MIC of the macrocyclic compounds and existing antibiotics.

Entry	Bacteria	Range of concentration (μg/ml)					Absorbance of suspension
		Compound **16**	Compound **17**	Streptomycin	Ampicillin	Rifampicin	
1	MTCC-619	10	10	2	–	10	0.620
2	MTCC-96	5	2	–	–	0.25	0.395
3	MTCC-722	1	2	–	–	0.25	0.765
4	MTCC-109	2	2	–	–	0.25	1.13

6.00–7.00 (8H, m, Ar-H), 7.80 (2H, s, CH=N in pyridine), 8.80 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 60.0 (2C, O–CH_2), 120.0, 121.0, 133.0, 137.0, 142.0, 143.0 (16C, Ar–C), 151.0 (2C, Ar–CH=N), 168.0 (2C, CH=N); mass spectrum, *m/z* 344 (7% M^+) Anal. found: C, 68.94; H, 4.89; N, 16.01%. Calcd for $C_{20}H_{16}N_4O_2$: C, 69.74; H, 4.68; N, 16.27%.

3.1.4. 13,14-Dihydro-12H-benzo[f]dipyrido[3,2-b:2,3-j][1,12,4,9]dioxadiaza-cyclopentadecine (**17**)

Yield 75%; mp 186; IR 1620, 1585, 1200, 3042w, 1446, 1400, 1380 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.20–2.30 (2H, m, C–CH_2–C), 3.92 (4H, t, O–CH_2), 7.80 (2H, s, CH=N in pyridine), 6.40–7.40 (8H, m, Ar-H), 8.60 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 30.2 (1C, C–CH_2–C), 67.4 (2C, O–CH_2), 122.7, 123.6, 133.5, 142.5 (16C, Ar-C), 155.2 (2C, Ar–CH=N), 170.2 (2C, CH=N); mass spectrum, *m/z* 358(4% M^+). Anal. found: C, 70.32; H, 5.12; N, 15.61%. Calcd for $C_{21}H_{18}N_4O_2$: C, 70.38; H, 5.06; N, 15.63%.

3.1.5. 21,22-Dihydrobenzo[i]dinaphtho[2,3-e:2,3-m][1,4,7,12]dioxadiaza-cyclotetradecine (**18**)

Yield 76%; mp 225; IR 1608.2, 1158.9, 3060.0w, 1497.4, 1449.9, 1372.9 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.37 (4H, s, O–CH_2), 7.00–7.50 (16H, m, Ar-H), 8.40 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 64.0 (2C, O–CH_2), 109.0, 124.0, 126.0, 126.0, 127.0, 133.0, 134.0, 140.0, 149.0 (26C, Ar-C), 167.0 (2C, CH=N); mass spectrum, *m/z* 442(9% M^+). Anal found: C, 81.89; H, 5.21; N, 6.07%. Calcd for $C_{30}H_{22}N_2O_2$: C, 81.43; H, 5.01; N, 6.33%.

3.1.6. 15,16-Dihydro-14H-benzo[f]binaphthol[2,3-b:2,3-j][1,12,4,9]dioxadiazacyclopenta decine (**19**)

Yield 79%; mp 216; IR 1610, 1208, 3040w, 1490, 1449, 1360 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.30–2.40 (2H, m, C–CH_2–C), 3.92 (4H, t, O–CH_2), 7.20–7.90 (16H, m Ar-H), 8.50 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 30.2 (1C, C–CH_2–C), 66.8 (2C, O–CH_2), 125.9, 126.2, 126.3, 127.0, 133.8, 148.5 (26C, Ar-C), 164.8 (2C, CH=N); mass spectrum, *m/z* 456 (15% M^+). Anal. found: C, 81.54; H, 5.30; N, 6.20%. Calcd for $C_{31}H_{24}N_2O_2$: C, 81.56; H, 5.36; N, 6.14%.

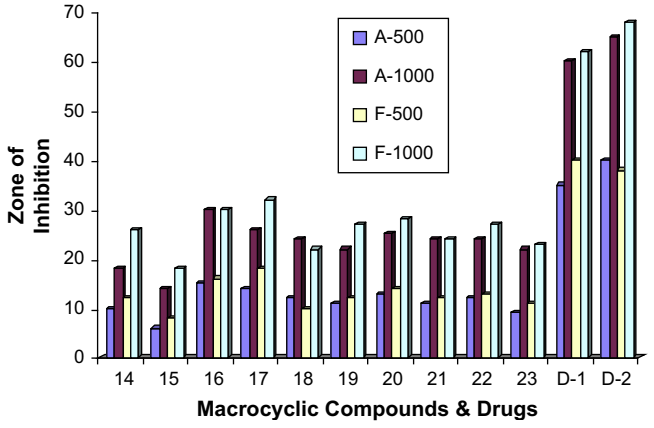


Fig. 1. Comparison of zone of inhibition of macrocyclic compounds and existing drug molecules against two different fungi.

3.1.7. 1,12-Dimethyl-6,7-dihydrotribenzo[e,i,m][1,4,7,12]dioxadiazacyclotetradecine (**20**)

Yield 84%; mp 184; IR 1622, 1141, 3040w, 1488, 1430, 1364 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.16 (6H, s, –CH_3), 4.02 (4H, s, O–CH_2), 6.72–7.46 (10H, m, Ar-H), 8.45 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 9.2 (2C, –CH_3), 64.6 (2C, O–CH_2–CH_2–O), 107.6, 123.4, 125.2, 128.8, 133.1, 133.9, 136.0, 137.7, 146.7 (18C, Ar-C), 164.8 (2C, CH=N); mass spectrum, *m/z* 370 (12% M^+). Anal. found: C, 77.72; H, 6.05; N, 7.60%. Calcd for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56%.

3.1.8. 7,19-Dimethyl-13,14-dihydro-12H-tribenzo[b,f,j][1,12,4,9]dioxadiazacyclopentadecine (**21**)

Yield 82%; mp 179; IR 1620, 1141, 3040w, 1487, 1430, 1363 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.18 (6H, s, –CH_3), 2.33–2.38 (2H, m, C–CH_2–C), 3.91–3.95 (4H, t, O–CH_2), 6.58–7.33 (10H, m, Ar-H), 8.41 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 19.4 (2C, –CH_3), 31.4 (1C, C–CH_2–C), 68.1 (2C, O–CH_2–CH_2–O), 107.9, 124.0, 125.8, 129.1, 133.5, 133.8, 136.7, 137.6, 146.7 (18C, Ar-C), 165.2 (2C, CH=N); mass spectrum, *m/z* 384 (7% M^+). Anal. found: C, 78.13; H, 6.28; N, 7.26%. Calcd for $C_{25}H_{24}N_2O_2$: C, 78.10; H, 6.29; N, 7.29%.

3.1.9. 2,11-Dimethyl-6,7-dihydrotribenzo[e,i,m][1,4,7,12]dioxadiazacyclotetradecine (**22**)

Yield 76%; mp 182; IR 1622, 1141, 3042w, 1488, 1431, 1363 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.27 (6H, s, –CH_3), 4.05 (4H, s, O–CH_2), 6.49–7.47 (10H, m, Ar-H), 8.42 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 21.0 (2C, –CH_3), 64.1 (2C, O–CH_2–CH_2–O), 113.7, 125.6, 129.4, 131.3, 133.0, 133.5, 133.8, 138.3, 150.2 (18C, Ar-C), 165.4 (2C, CH=N); mass spectrum, *m/z* 370(14% M^+). Anal. found: C, 77.72; H, 6.05; N, 7.60%. Calcd for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56%.

3.1.10. 8,18-Dimethyl-13,14-dihydro-12H-tribenzo[b,f,j][1,12,4,9]dioxadiaza cyclopentadecine (**23**)

Yield 78%; mp 176; IR 1622, 1140, 3040w, 1487, 1432, 1362 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.24 (6H, s, –CH_3), 2.33–2.39 (2H, m, C–CH_2–C), 3.94–3.98 (4H, t, O–CH_2), 6.64–7.62 (10H, m, Ar-H), 8.48 (2H, s, CH=N); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 21.0 (2C, –CH_3), 30.3 (1C, C–CH_2–C), 68.0 (2C, O–CH_2–CH_2–O), 113.7, 126.1, 129.7, 131.2, 133.1, 133.6, 134.8, 138.6, 150.0 (18C, Ar-C), 166.0 (2C, CH=N); mass spectrum, *m/z* 384 (11% M^+). Anal. found: C, 78.13; H, 6.28; N, 7.26%. Calcd for $C_{25}H_{24}N_2O_2$: C, 78.10; H, 6.29; N, 7.29%.

3.2. Antimicrobial testing by agar diffusion

Antimicrobial testing was done by cup plate method [37]. 27 ml of molten agar was added to sterile Petri dishes and allowed to solidify for 1 h. Then 50 ml of the 24 h culture of a test organism was spread evenly onto the agar plate with the sterile cotton swab. Six millimetre wide bores were made on the agar using a borer. The

solutions of the macrocyclic compounds were added into 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 h. 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.

3.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration [38] was determined by liquid dilution method. Stock solutions of macrocyclic compounds with 2.5, 5, 10, 20, 50 and 100 μg/ml concentrations were prepared with appropriate solvent. The solutions of standard drugs like Streptomycin, Ampicillin and Rifampicin 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 h 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, Ampicillin and Rifampicin for comparison.

3.4. 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 [39] 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 [40].

% 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.

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