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Original article

Synthesis, Raman, FT-IR, NMR spectroscopic data and antimicrobial activity of mixed aza-oxo-thia macrocyclic compounds

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Abstract

Mixed aza-oxo-thia macrocyclic ligands 1,3,5,11,13,15-hexaaza-6,10,16,20-tetraoxo-8,18-dithia-2,3,4:12,13,14-dipyridine cyclocosane **(L_1)**; 1,3,5,12,14,16-hexeaza-6,11,17,22-tetraoxo-8,9,19,20-tetrathia-2,3,4:13,14,15-dipyridine cyclodocosane **(L_2)**; 1,3,5,13,15,17-hexaaza-6,12,18,24-tetraoxo-9,21-dithia-2,3,4:14,15,16-dipyridine cyclotetracosane **(L_3)** and 1,3,5,14,16,18-hexaaza-6,13,19,26-tetraoxo-9,10,22,23-tetrathia-2,3,4:15,16,17-dipyridine cyclohexacosane **(L_4)** were synthesised. The structural features of the ligands have been studied by elemental analyses, Raman, IR, ^{1}H and ^{13}C NMR spectroscopy. The antimicrobial activities of the ligands were evaluated using disk diffusion method in dimethyl sulfoxide (DMSO) as well as the minimal inhibitory concentration (MIC) dilution method, against nine bacteria. The obtained results from disk diffusion method were assessed in side-by-side comparison with those of penicillin G, ampicillin, cefotaxime, vancomycin, ofloxacin, and tetracycline well known antibacterial agents. The results from dilution procedure were compared with gentamycin as antibacterial and nystatin as antifungal. The antifungal activities are reported on five yeast cultures namely *Candida albicans*, *Kluyveromyces fragilis*, *Rhodotorula rubra*, *Debaryomyces hansenii*, and *Hanseniaspora guilliermondii*, and the results are referenced with nystatin, Ketoconazole, and clotrimazole, commercial antifungal agents. In most cases, the compounds show broad-spectrum (Gram$^+$ and Gram$^-$ bacteria) activities that were more active or equipotent to the antibiotic and antifungal agents in the comparison tests.

Keywords: Broad-spectrum; Clotrimazole; Disk diffusion; Nystatin; Raman

1. Introduction

For several decades, the design and synthesis of macro-hetero-multi-donor ligands have constituted one of the largest areas of research in organic and coordination chemistry [1–5]. In certain cases, nature prefers macrocyclic derivatives for many fundamental biological functions such as photosynthesis, storage and transport of oxygen in mammalian and other respiratory systems. Having various donor centres, macrocycles offer exciting possibilities to construct novel supramolecular assemblies that are capable of performing highly specific molecular functions. For instant, the precise molecular recognition between these compounds and their guests, mostly transition metal ions or biomolecules (nucleic acids, proteins…), provides a good opportunity for studying key aspects of supramolecular chemistry, which are also significant in a variety of disciplines including bioorganic chemistry, biocoordination chemistry, biology, medicine and related science and technology [6–8]. Chemically, multi-donor ligands and particularly mixed donor atoms of these ligands are important because of great availability as ligands due to the presence of several potential donor centres and their flexibility to bind with biomolecules or to coordinate with various metal ions. Among these, the N–S donor macrocycles also have theoretical interest, as they are capable of furnishing an environment of controlled geometry and ligand field strength [9–11].

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Table 1
Prominent IR and Raman bands for the compound

Compounds	FT-IR (cm^{-1})	Raman (cm^{-1})
(L_1)	3439 ν(H–O–H), 3313 ν(N–H), 3117 ν(C–H)$_{py}$, 2963, 2927 ν(C–H), 1692, 1659 ν(C=O), 1640 δ(N–H), 1622 ν(C–C)$_{py}$, 1553 ν(C–N)$_{py}$, 1388–1310 [ν(C–N)$_{py}$, ν(C–C)$_{py}$], 976, 780 ω(C–H)$_{py}$, 712 ν(C–S), 565, 491, 448, 413	3084, 3051 ν(C–H)$_{py}$, 2972, 2921 ν(C–H), 1645 ν(C=O), 1624 ν(C–C)$_{py}$, 1557 ν(C–N)$_{py}$, 1314 [ν(C–N)$_{py}$, ν(C–C)$_{py}$], 1130, 1000, 975, 808 ω(C–H)$_{py}$, 711 ν(C–S), 603, 563, 545, 441, 356, 289, 222
(L_2)	3405 ν(H–O–H), 3346, 3211 ν(N–H), 3096, 3058 ν(C–H)$_{py}$, 2994, 2971 ν(C–H), 1671 ν(C=O), 1647 δ(N–H), ν(C–C)$_{py}$, 1571 ν(C–N)$_{py}$, 1404–1296 [ν(C–N)$_{py}$, ν(C–C)$_{py}$], 922, 773 ω(C–H)$_{py}$, 722 ν(C–S), 522 ν(S–S), 464, 444	3097, 3042 ν(C–H)$_{py}$, 2941, 2927 ν(C–H), 1686, 1650 ν(C=O), 1617 ν(C–C)$_{py}$, 1568 ν(C–N)$_{py}$, 1490–1306 [ν(C–N)$_{py}$, ν(C–C)$_{py}$], 987, 923, 763 ω(C–H)$_{py}$, 687 ν(C–S), 580, 566, 544, 522 ν(S–S), 356, 260
(L_3)	3444 ν(H–O–H), 3331, 3217 ν(N–H), 3077 ν(C–H)$_{py}$, 2965 ν(C–H), 1708, 1673 ν(C=O), 1644 δ(N–H), 1626 ν(C–C)$_{py}$, 1548 ν(C–N)$_{py}$, 1403–1301 [ν(C–N)$_{py}$, ν(C–C)$_{py}$], 1168, 974, 777 ω(C–H)$_{py}$, 727 ν(C–S), 683, 671, 560, 445	3078, 3041 ν(C–H)$_{py}$, 2919 ν(C–H), 1648 ν(C=O), 1622 ν(C–C)$_{py}$, 1561 ν(C–C)$_{py}$, 1408–1298 [ν(C–N)$_{py}$, ν(C–C)$_{py}$], 980, 928, 758, 668 ν(C–S), 558, 357, 208
(L_4)	3398 ν(H–O–H), 3370, 3216 ν(N–H), 3062 ν(C–H)$_{py}$, 2985 ν(C–H), 1657 ν(C=O), 1649 δ(N–H), 1622 ν(C–C)$_{py}$, 1567 ν(C–N)$_{py}$, 1416 [ν(C–N)$_{py}$, ν(C–C)$_{py}$], 1164, 1137, 819, 795 ω(C–H)$_{py}$, 715 ν(C–S), 690, 589, 564, 515 ν(S–S), 483	3095, 3041 ν(C–H)$_{py}$, 2955, 2922 ν(C–H), 1644 ν(C=O), 1622 ν(C–C)$_{py}$, 1562 ν(C–C)$_{py}$, 1410–1304 [ν(C–N)$_{py}$, ν(C–C)$_{py}$], 763, 691, 659, 638 ν(C–S), 565, 546, 510 ν(S–S), 362, 210

ν, stretching; δ, bending; ω, out-of-plane wagging; and py, pyridine-ring.

2. Experimental protocols

2.1. Chemistry

All chemicals and solvents were reagent grade and were used as purchased without further purification. Melting points were determined using an Electro-thermal 9100 melting-point apparatus. Analytical data were obtained with a Thermo Finnigan Flash EA 1112 analyser. FT-IR spectra were recorded as KBr pellets on a Jasco FT/IR-600 Plus spectrometer. FT-Raman spectra were obtained from powdered samples placed in a Pyrex tube using the Bruker RFS 100/S spectrometer in the range 4000–20 cm^{-1}. The 1064 nm line, provided by a near infrared Nd:YAG air-cooled laser was used as excitation line. The output laser power was set to 180–200 mW. Routine ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded at ambient temperature in DMSO-d_6. Chemical shifts (δ) are expressed in units of parts per million relative to TMS. The analytical data and physical properties are summarized for each experiment and the spectral data are presented in Tables 1–3. The synthetic pathway for the compounds is shown in Scheme 1.

2.1.1. Synthesis

*2.1.1.1. 1,3,5,11,13,15-hexaaza-6,10,16,20-tetraoxo-8,18-dithia-2,3,4:12,13,14-dipyridine cyclocosane **(L_1)***. Centrifuged hot ethanolic solution (10 mL, absolute) of 2,6-pyridinediamine (2.25 g, 20.64 mmol) was mixed with hot ethanolic solution of (10 mL) of thiodiglycolic acid (3.0 g, 20.64 mmol) in the presence of few drops of concentrated HCl. The solution mixture was refluxed for 6–8 h at 85 °C. The resulting reaction mixture was refrigerated overnight. The light yellow crystalline solid was formed, which was filtered, washed with cold EtOH and dried under vacuum (3.80 g, 82%). M.p. 197 °C. Found (calculated) **(L_1)(H_2O)**, $C_{18}H_{20}N_6O_5S_2$: C, 46.15 (46.55); H, 4.27 (4.31); N, 18.33 (18.10); S, 13.88 (13.79).

The other ligands were prepared in a similar manner to the ligand **(L_1)** and the results are presented as following.

*2.1.1.2. 1,3,5,12,14,16-hexeaza-6,11,17,22-tetraoxo-8,9,19,20-tetrathia-2,3,4:13,14,15-dipyridine cyclodocosane **(L_2)***. Hot ethanolic solution (10 mL) of 2,6-pyridinediamine (2.25 g, 20.64 mmol) was reacted with ethanolic solution of (10 mL) of dithiodiacetic acid (3.64 g, 20.64 mmol) in the presence of few drops of HCl. The off white crystalline solid was obtained (4.31 g, 82%). M.p. 131–136 °C. Found (calculated) **(L_2)(H_2O)$_3$**, $C_{18}H_{24}N_6O_7S_4$: C, 37.78 (38.30); H, 4.67 (4.26); N, 14.56 (14.89); S, 22.13 (22.69).

*2.1.1.3. 1,3,5,13,15,17-hexaaza-6,12,18,24-tetraoxo-9,21-dithia-2,3,4:14,15,16-dipyridine cyclotetracosane **(L_3)***. Hot ethanolic solution (10 mL) of 2,6-pyridinediamine (2,25 g,

Table 2
^{1}H chemical shift values (ppm) for the **(L_1–L_4)** compounds

Compound	a(8H)	b(8H)	d(4H)	e(2H)	NH(4H)
(L_1)	3.30 (s)	–	5.7 (d, J = 8.0 Hz)	7.34 (t, J = 8.0 Hz)	9.80–7.50
(L_2)	3.60 (s)	–	5.79 (d, J = 8. 06 Hz)	7.31 (t, J = 8.06 Hz)	10.60–6.50
(L_3)	2.70 (t, J = 7.17 Hz)	2.48 (t, J = 7.17 Hz)	5.67 (d, J = 7.80 Hz)	7.08 (t, J = 7.80 Hz)	7.00–5.80
(L_4)	2.89 (t, J = 6.92 Hz)	2.58 (t, J = 6.92 Hz)	5.71(d, J = 7.90 Hz)	7.16 (t, J = 7.90 Hz)	9.10–6.20

For the (a–e) definition, please refer to Figs. 4, 5 and Scheme 1.

Table 3
Observed and theoretical ^{13}C chemical shift values (ppm) for the **(L_1–L_4)** compounds

Compound	a	b	C=O	c	d	e
(L_1)	35.40 (35.70)	–	172.85 (168.20)	155.60 (147.70)	95.49 (111.70)	142.48 (141.40)
(L_2)	42.67 (38.00)	–	172.28 (168.20)	154.95 (147.70)	95.41 (111.70)	143.18 (141.40)
(L_3)	26.65 (28.10)	35.11 (35.90)	173.90 (182.00)	157.68 (147.70)	95.18 (111.70)	139.31 (141.40)
(L_4)	33.54 (30.80)	34.29 (34.80)	173.63 (172.70)	156.44 (147.70)	95.09 (111.70)	140.62 (141.40)

For the (a–e) and C=O definition, please refer to Figs. 4, 5 and Scheme 1.

20.64 mmol) was reacted with ethanolic solution of (10 mL) of 3,3′-thiodipropionic acid (3.56 g, 20.64 mmol) in the presence of few drops of HCl. Off white crystalline solid was obtained (4.38 g, 85%). M.p. 179 °C. Found (calculated) **(L_3)(H_2O)$_2$**, $C_{22}H_{30}N_6O_6S_2$: C, 48.12 (49.10); H, 5.10 (5.58); N, 14.94 (15.61); S, 11.21 (11.89).

*2.1.1.4. 1,3,5,14,16,18-hexaaza-6,13,19,26-tetraoxo-9,10,22,23-tetrathia-2,3,4:15,16,17-dipyridine cyclohexacosane **(L_4)***. Hot ethanolic solution (10 mL) of 2,6-pyridinediamine (2,25 g, 20.64 mmol) was reacted with ethanolic solution of (10 mL) of 3,3′-dithiodipropionic acid (4.20 g, 20.64 mmol) in the presence of few drops of HCl. The off white crystalline solid was obtained (4.65 g, 80%). M.p. 123–125 °C. Found (calculated) **(L_3)(H_2O)$_2$**, $C_{22}H_{30}N_6O_6S_4$: C, 44.34 (43.85); H, 4.55 (4.98); N, 14.21 (13.95); S, 20.87 (21.26).

2.2. Pharmacology

The antimicrobial activities are evaluated against Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* La 2971) and Gram-negative (*Escherichia coli* ATCC 11230, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Enterobacter aerogenes* ATCC 13048) bacteria and the yeast cultures (*Candida albicans* ATCC 10231, *Kluyveromyces fragilis* NRRL 2415, *Rhodotorula rubra* DSM 70403, *Debaryomyces hansenii* DSM 70238 and *Hanseniaspora guilliermondii* DSM 3432) using both the disk diffusion and the dilution methods.

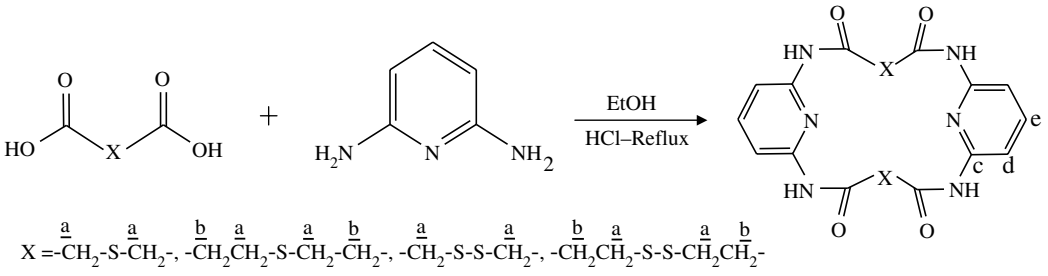
2.2.1. Methods

2.2.1.1. Disk diffusion method. Sterilised antibiotic discs (6 mm) were used following the literature procedure [12,13]. Fresh stock solutions of the ligands were prepared in DMSO according to the needed concentrations for experiments. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO as the same procedures as used in the experiments. All the bacteria were incubated at 30 °C for 24 h in Nutrient Broth. The yeasts were incubated in Malt Extract Broth for 48 h. The discs injected with solutions were placed on the inoculated agar and incubated at 35 °C (24 h) and at 25 (72 h) for bacteria and yeast, respectively. In each case triplicate tests were performed and the average was taken as the final reading.

2.2.1.2. Dilution method. Screening was performed following the procedure outlined in the Manual of Clinical Microbial [14]. All the bacteria were incubated and activated at 30 °C for 24 h inoculation into Nutrient Broth and the yeasts were incubated in Malt Extract Broth for 48 h. The compounds were dissolved in DMSO and then diluted using cautiously adjusted Mueller Hinton Broth. Two-fold serial concentrations of the compounds were employed to determine the (MIC) ranging from 200 to 1.56 μg mL^{-1}. In each case triplicate tests were performed and the average was taken as the final reading.

2.2.2. Biological data

Standardised samples of penicillin G (blocking the formation of bacterial cell walls, rendering bacteria unable to multiply and spread), ampicillin (preventing the growth of Gram-negative bacteria), cefotaxime (acting against most Gram-negative enteric bacteria), vancomycin (interfering with the construction of cell walls in bacteria), ofloxacin (entering the bacterial cell and inhibiting DNA-gyrase, preventing the bacteria from reproducing), tetracyclines (inhibiting the protein synthesis), nystatin (binding to sterols in the fungal cellular membrane, altering the permeability and allowing leakage of the cellular contents), Ketoconazole (inhibiting the growth of fungal organisms by interfering with the formation of the fungal cell wall), and clotrimazole (killing fungi and yeasts by interfering with their cell membranes). Mueller Hinton Media, Nutrient



Scheme 1. Synthetic pathway for preparation of macrocyclic compounds **(L_1–L_4)**.

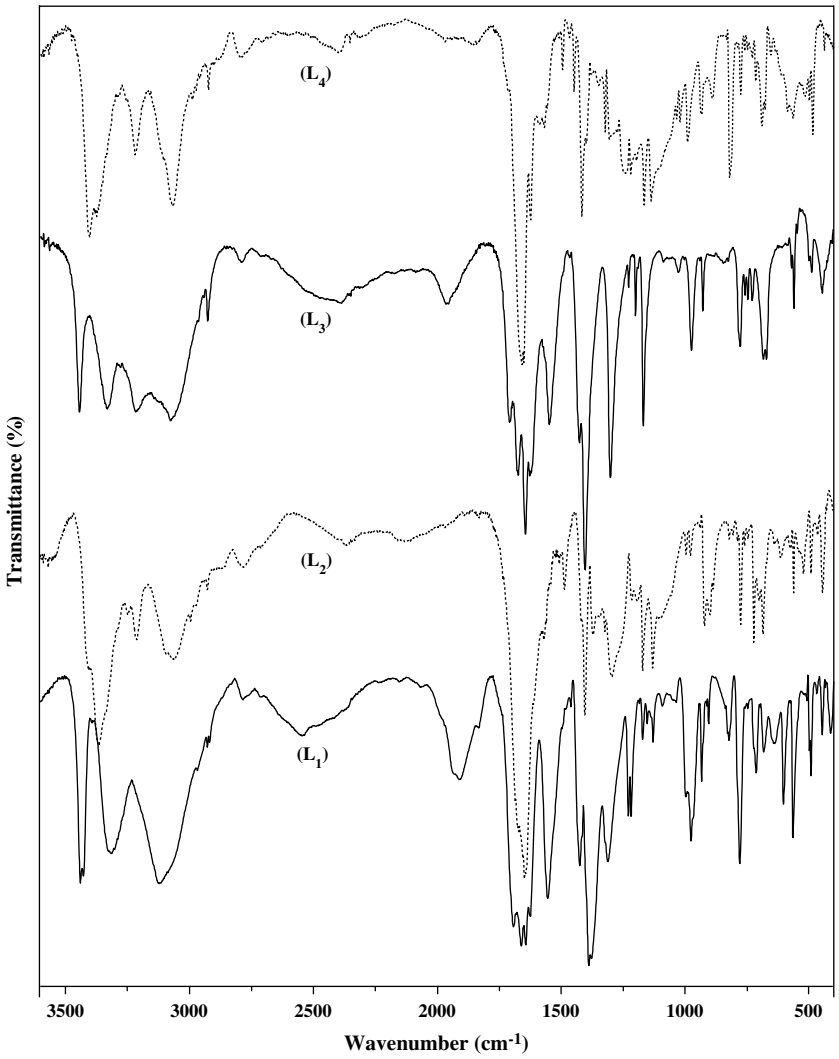


Fig. 1. FT-IR spectrum of **(L_1–L_4)** in the 3600–400 cm^{-1} region.

Broth and Malt Extract Broth are purchased from Difco and yeast extracts is obtained from Oxoid.

3. Results and discussion

3.1. Vibrational spectra

The present vibrational spectra can be discussed in terms of three characteristic wave regions: 3500–2800 cm^{-1} corresponding to ν(N–H), $\nu(C–H)_{py}$ and ν(C–H) characteristic stretching modes, 1800–750 cm^{-1} belongs to ν(CO), $\nu(C–N)_{py}$, $(C–C)_{py}$, $\delta(C–H)_{py}$, δ(N–H) and 750–400 cm^{-1} frequencies regions and 750–400 cm^{-1}, due to the ν(C–S) and ν(S–S) characteristic stretching modes, particularly in Raman spectra. Prominent Raman and IR band values are presented in Table 1. Appearance of two strong bands in the region 3450–3330 cm^{-1} in the IR spectra may be due to the presence of lattice water ν(H–O–H) (antisymmetric and symmetric), which was also confirmed by elemental analysis [15]. The bands corresponding to free –COOH and $-NH_2$ groups are not observed in the IR spectra of the **(L_1–L_4)** ligands, which suggest the complete condensation of the amine group of 2,6-pyridinediamine with corresponding dicarboxylic acids. Medium to strong bands observed in the region 3300–3200 cm^{-1} in the IR spectra of these macrocyclic compounds, may be assignable to ν(N–H) of the secondary amino groups. Due to nonplanar characteristic behaviour of the compounds in the solid state, these bands could be observed as multiple signals. These lines have extremely weak Raman intensity. The bending vibrations for δ(H–O–H) and δ(N–H) groups in IR spectra are observed in the region 1640 and 1500 cm^{-1}, respectively [15]. In both Raman and IR spectra, the characteristic ν(CH) modes of ring residues and aliphatic groups are observed in the wave region 3100–2900 cm^{-1}. Several new bands are appeared, which could be assignable to at 1710–1650 ν(CO) characteristic of amide groups, at *ca.* 1640 δ(N–H), 1620 $\nu(C–C)_{py}$, 1570–1550 $[\delta(N–H)\text{-IR} + \nu(C–N)_{py} + \nu(C–N)]$, and 800–760 cm^{-1} $[\delta(C–H)_{py} + \delta(CO)]$ (Figs. 1 and 2).

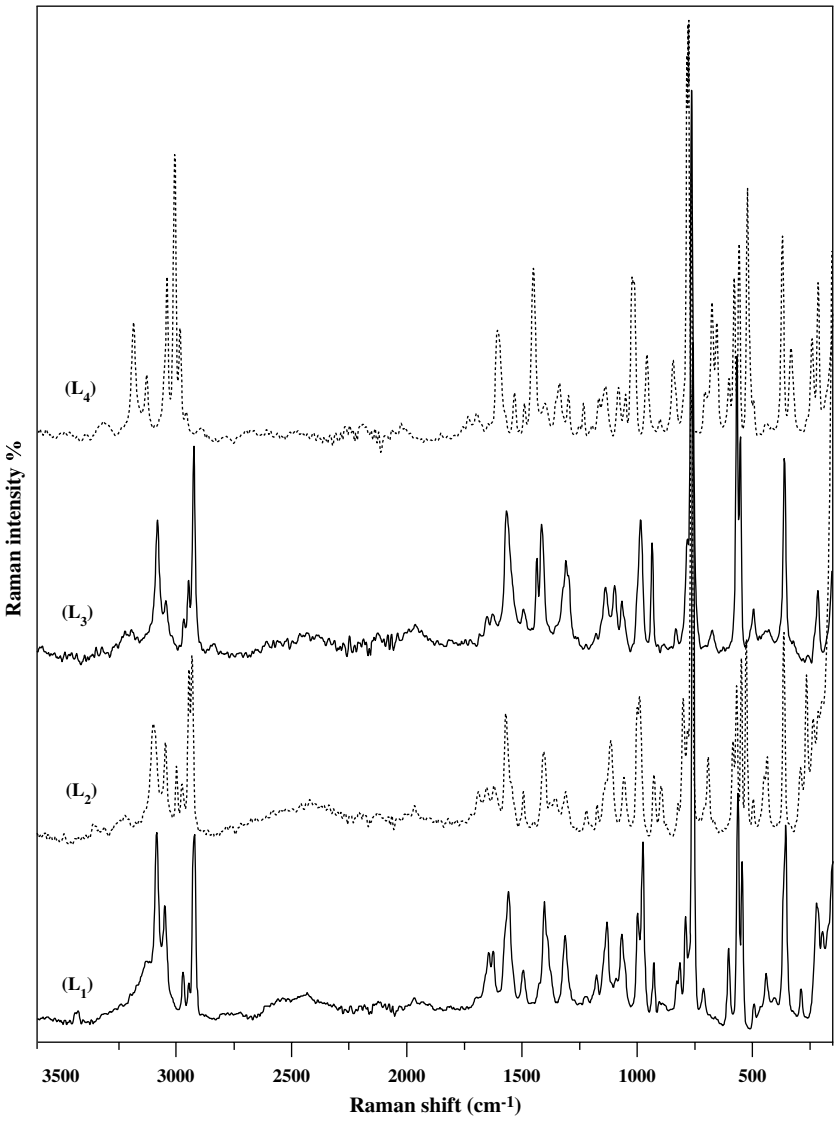


Fig. 2. FT-Raman spectrum of **(L_1–L_4)** in the 3500–150 cm^{-1} region.

The vibrational frequency of an isolated S_2 molecule in the inert gas matrix (mat) is measured at 718 cm^{-1} [16]. The $\nu(S_2)$ in the free-state (solid doped in KCl) is 623 cm^{-1}. In the Raman spectra, ν(C–S) and ν(S–S) modes in the (C–S–C) and (C–S–S–C) moieties are very characteristic giving rise to a medium to strong stretching bands in the wave region 710–640 and 522–510 cm^{-1}, respectively [17,18]. The strong bands at 522 of **(L_2)** and 510 cm^{-1} of **(L_4)** show characteristic ν(S–S) stretching modes for these macrocycles (Fig. 3). The assignments are supported by the fact that the other relevant bands at this region remain almost unchanged among these compounds. Because of the non-polar character of the (C–S) and (S–S) bonds, their counterpart in IR is quite weak (Fig. 1).

3.2. Nuclear magnetic resonance

The ^{1}H NMR spectra pattern has changed significantly due to the cyclic formation compared with 2,6-pyridinediamine and corresponding dicarboxylic acids. The bands corresponding to –COOH (*ca.* 11 ppm) and $-NH_2$ (at 5.54 ppm) groups are not observed in the ^{1}H NMR spectra of the **(L_1–L_4)** compounds, instead the amino proton signals were appeared as broad bands in the region 8.5–7.50 ppm. The low field position as well as broadness of NH protons could be attributed to the combination of deshielding and weak hydrogen bonding due to amide and sulphur groups. Full NMR spectra data (^{1}H and ^{13}C) and their assignments are presented in Tables 2 and 3. Comparisons of these chemical shift values with the starting materials tend to support the complete condensation of the amine and carboxylic acid groups, which was also indicated by vibrational spectra.

In conclusion, presented structures in Figs. 4 and 5 are in best accord with the experimental data obtained from the analytical, vibrational and nuclear magnetic resonance spectra.

3.3. Antimicrobial activity

The results concerning *in vitro* antimicrobial activities of the macrocycles together with the inhibition zone (mm) and

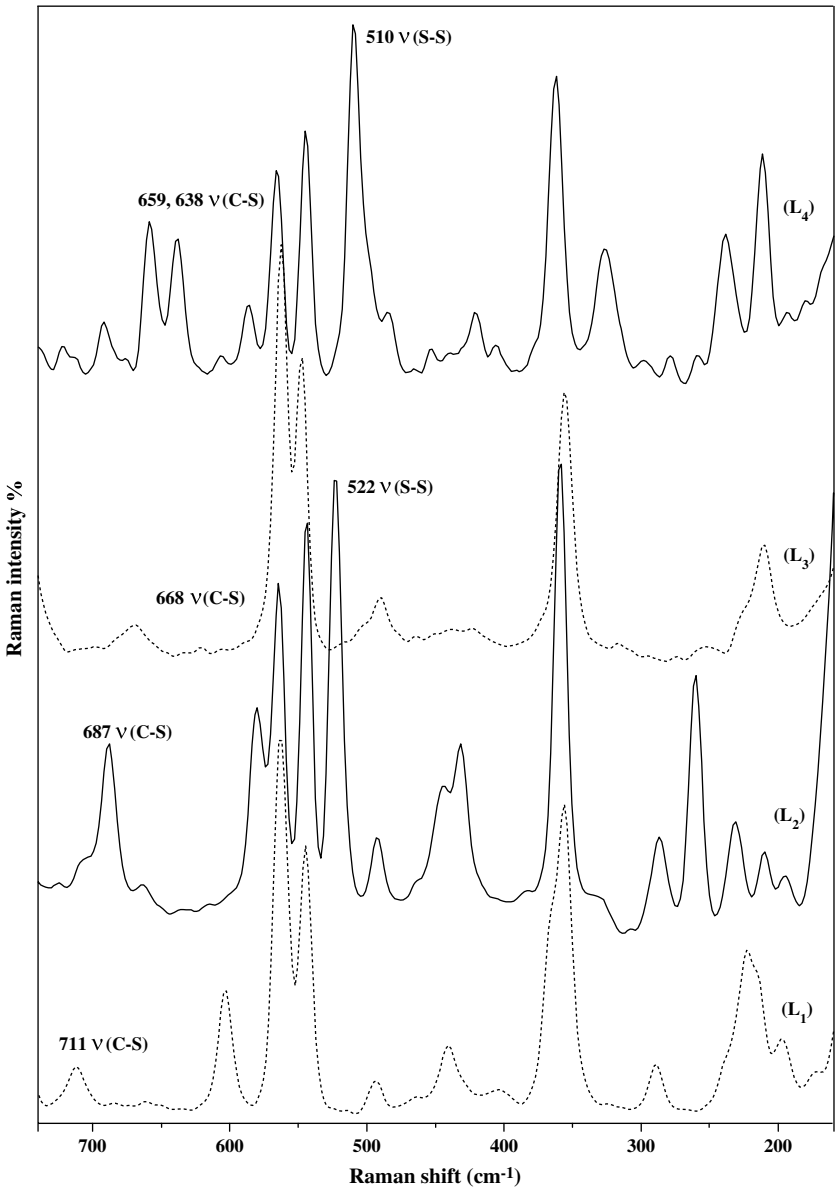


Fig. 3. FT-Raman spectrum of **(L_1–L_4)** in the 740–160 cm^{-1} region.

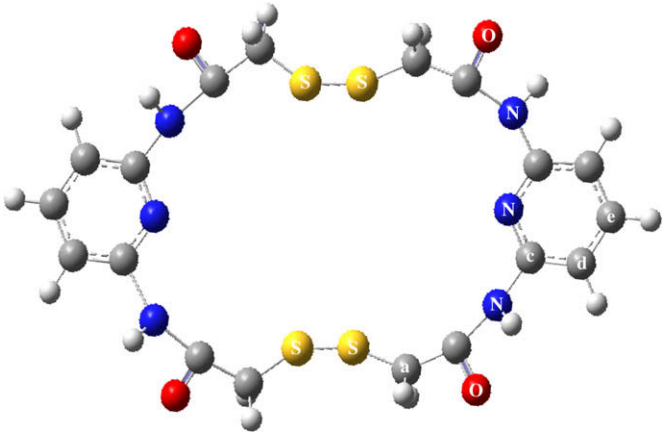


Fig. 4. Structure of 1,3,5,12,14,16-hexeaza-6,11,17,22-tetraoxo-8,9,19,20-tetrathia-2,3,4 13, 14,15-dipyridine cyclodocosane **(L_2)**.

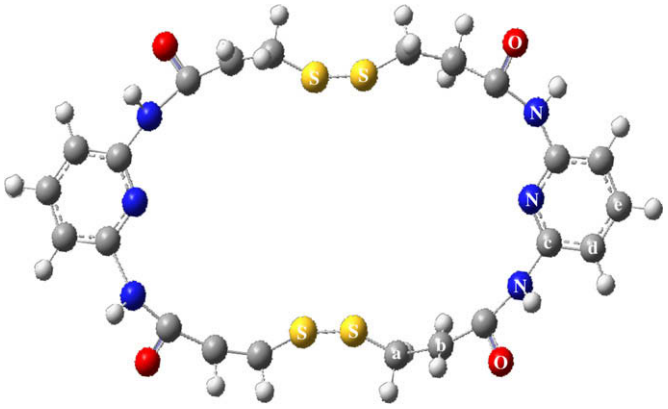


Fig. 5. Structure of 1,3,5,14,16,18-hexaaza-6,13,19,26-tetraoxo-9,10,22,23-tetrathia-2,3,4:15,16,17-dipyridine cyclohexacosane **(L_4)**.

Table 4
In vitro antimicrobial activity of the compounds and the standard reagents (inhibition zone, mm)

Microorganisms	Compounds												
	(L_1)	**(L_2)**	**(L_3)**	**(L_4)**	P10	AMP	CTX	VA	OFX	TE	NY	KET	CLT
Escherichia coli	10.0	14.0	11.0	16.0	18	12	10	22	30	28	–	–	–
Staphylococcus aureus	14.0	14.0	13.0	15.0	13	16	12	13	24	26	–	–	–
Klebsiella pneumoniae	15.0	15.0	14.0	17.0	18	14	13	22	28	30	–	–	–
Pseudomonas aeruginosa	16.0	14.0	15.0	17.0	14	12	14	18	30	25	–	–	–
Proteus vulgaris	11.0	11.0	10.0	11.0	36	32	32	34	28	22	–	–	–
Bacillus cereus	12.0	14.0	11.0	15.0	10	16	18	20	28	26	–	–	–
Mycobacterium smegmatis	10.0	10.0	10.0	10.0	15	21	11	20	32	24	–	–	–
Listeria monocytogenes	12.0	13.0	13.0	15.0	10	12	16	26	30	28	–	–	–
Micrococcus luteus	12.0	16.0	13.0	15.0	8	10	54	10	44	34	–	–	–
Candida albicans	17.0	21.0	16.0	22.0	–	–	–	–	–	–	18	16	18
Kluyveromyces fragilis	20.0	19.0	21.0	22.0	–	–	–	–	–	–	18	22	16
Rhodotorula rubra	15.0	19.0	17.0	22.0	–	–	–	–	–	–	20	21	15
Hanseniaspora guilliermondii	20.0	20.0	21.0	22.0	–	–	–	–	–	–	21	24	22
Debaryomyces hansenii	18.0	21.0	19.0	22.0	–	–	–	–	–	–	16	14	18

P10, penicillin G (10 units); AMP, ampicillin (μg); CTX, cefotaxime (μg); VA, vancomycin (μg); OFX, ofloxacin (5 μg); TE, tetracycline (30 μg); NY, nystatin (μg); KET, Ketoconazole (μg); and CLT, clotrimazole (10 μg).

(MIC) values of compared antibiotic and antifungal are listed in Tables 4 and 5. All the compounds tested exhibit strong or moderate antimicrobial activity. Of all the test compounds attempted, **(L_2)** and **(L_4)** showed slightly higher activities against most Gram-positive and Gram-negative bacteria and as well as yeast cultures. The MIC values in Table 5 also indicate that all the compounds tested exhibit moderate to strong antimicrobial activity on the tested microorganisms. Once again the data indicate that the **(L_2)** and **(L_4)** macrocycles have slightly stronger activity against most Gram-positive and Gram-negative bacteria compared with **(L_1)** and **(L_3)**, but all the tested compounds have strong activity against the yeast cultures. For instance, **(L_4)** showed superior activity (MIC = 1.56 μg mL^{-1}) against all the tested cultures.

The inhibition activity seems to be governed in certain degree by the percentage amount of the sulphur presence in these compounds, because the **(L_2)** and **(L_4)** macrocycles are the most active against the most tested microorganisms, compared to the monosulphur macrocycles. In classifying the antibacterial activity as Gram-positive or Gram-negative, it would generally be expected that a much greater number would be active against Gram-positive than Gram-negative bacteria. However, in this study, the compounds are active against both types of the bacteria and as well as active against yeasts, which may indicate a broad-spectrum affect. The results of our study indicate that the compounds have the potential to generate novel antimicrobial properties by displaying moderate to high affinities for most of the receptors.

Table 5
In vitro antimicrobial activity (MIC, μg mL^{-1}) of the compounds and standard reagents

Microorganisms	Compounds					
	(L_1)	**(L_2)**	**(L_3)**	**(L_4)**	GEN	NYS
Escherichia coli	25	12.5	25	6.25	6.25	–
Staphylococcus aureus	12.5	12.5	12.5	6.25	25	–
Klebsiella pneumoniae	6.25	6.25	6.25	6.25	6.25	–
Bacillus cereus	6.25	12.5	6.25	6.25	6.25	–
Micrococcus luteus	25	25	25	25	25	–
Proteus vulgaris	12.5	12.5	25	12.5	6.25	–
Mycobacterium smegmatis	25	25	25	25	12.5	–
Listeria monocytogenes	12.5	12.5	12.5	12.5	12.5	–
Pseudomonas aeruginosa	12.5	6.25	12.5	12.5	6.25	–
Kluyveromyces fragilis	6.25	3.125	6.25	1.56	–	6.25
Rhodotorula rubra	3.125	3.125	6.25	1.56	–	6.25
Candida albicans	6.25	3.125	3.125	1.56	–	3.125
Hanseniaspora guilliermondii	3.125	3.125	1.56	1.56	–	3.125
Debaryomyces hansenii	3.125	3.125	3.125	1.56	–	12.5

GEN, gentamycin; NYS, nystatin.

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