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Original article

Structural characterization and antimicrobial activity of 1,3-bis(2-benzimidazyl)-2-thiapropane ligand and its Pd(II) and Zn(II) halide complexes

Naz M. Agh-Atabay [a,*], Basaran Dulger [b], Fahrettin Gucin [a]

[a] *Department of Chemistry, Faculty of Arts and Science, Fatih University, 34500 Buyukcekmece, Istanbul, Turkey*
[b] *Department of Biology, Faculty of Arts and Science, Canakkale Onsekiz Mart University, Canakkale, Turkey*

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Abstract

1,3-Bis(2-benzimidazyl)-2-thiapropane(L) forms 5-coordinate square pyramidal and 4-coordinate tetrahedral, monometallic complexes with $PdCl_2$ and ZnX_2 (X = Cl, Br, I), respectively. In the palladium complex, the ligand acts as a chelating tridentate, through two of the nitrogen atoms in the imidazole ring and the sulfur atom of the bridging group together with two chloride ions forming a rare five coordinate complex. In the zinc halide complexes, the ligand acts as chelating bidentate, *via* two of the nitrogen atoms combined with two halide ions giving common tetrahedral complexes. The ligand and its complexes are characterized by analytical data and spectroscopic methods such as FT-Raman, FT-IR (mid-IR, far-IR), ^1H and ^{13}C NMR. Their antimicrobial activities are evaluated by the minimal inhibitory concentration (MIC) against 10 bacteria, each with multiple, fresh clinical isolates (10–15), and the results are compared with those of ampicillin, ciprofloxacin, cefazolin, ofloxacin, and piperacillin antibacterial agents. The compound's antifungal activities are reported on *Candida albicans*, *Candida utilis*, and *Cryptococcus neoformans* yeasts, each with multiple isolates (10), and the results are referenced with amphotericin-B, fluconazole and flucytosine antifungal agents. In most cases, the compounds show broad-spectrum ($Gram^+$ and $Gram^-$) activities that are either, more active, or equipotent to, the antibiotic and antifungal agents in the comparison tests.

Keywords: Amphotericin-B; Antifungal; Antimicrobial; Chelating; Equipotent; Fluconazole; Monometallic; Piperacillin; Pyramidal; Tridentate

1. Introduction

Due to the following facts imidazole and benzimidazole derivatives together with their transition metal complexes are been extensively investigated. Their structural similarities with the common pyrimidine and purine type nucleobases, they attract a great deal of interest in the field of biological investigations particularly on the nucleic acid related studies. They are the key components in a great many bioactive compounds of both natural and synthetic origin. Their inhibitory properties as regards the replication of polioviruses, adenosine deaminase, and casein kinase have been demonstrated [1]. While others are important potent antiviral agents [2,3], many are active components of biocides such as fungicides, and insecticides [4]. For instance [2-(4′-thiazolyl) benzimidazole], known as thiabendazole, and its several coordination compounds aroused considerable interest in biology and medicine due to their strong antimicrobial activities [5–8]. Other bis-benzimidazole derivatives, such as 1,2-bis-(2-benzimidazyl)-1,2-ethanediol, 1,3-bis(2-benzimidazyl)-2-thiapropane and 1,5-bis(2-benzimi-dazyl)-3-thiapentane also have strong antimicrobial characteristic against several microorganisms [9].

In addition to their biological importance, they form stable complexes with various transition metal ions [10–12]. The interaction of these ions with biologically active ligands, for instance in drugs, is a subject of great interest. Some of the biological active compounds do act *via* chelation, but for most little is known about how metal coordination influences their activity. Although it is believed that they react selectively towards certain biological systems [13,14]. In some cases the

* Corresponding author. Tel.: +90 212 889 0810; fax: +90 212 889 0832.
E-mail address: natabay@fatih.edu.tr (N.M. Agh-Atabay).

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highest activity is associated with a metal atom [9,15]. The metal oxidation state, the type and number of donor atoms, as well as their relative disposition within the ligand are major factors determining structural and activity relationship.

In this work, the palladium chloride and zinc halide complexes with 1,3-bis(2-benzimidazyl)-2-thiapropane(L) ligand are reported. Their antibacterial and antifungal activities are evaluated by the minimum inhibitory concentration (MIC) against 10 bacteria and three yeasts. Herein, we discuss the difference of metal ion complexes as well as the free ligand in their structural–biological activity relationship.

2. Chemistry

Ligand (L) is prepared by literature procedures [9,16]. Syntheses of the palladium and zinc halide complexes are carried under reflux in absolute ethanol. Full general procedures for preparation of these compounds are given in the experimental and the reactions sequence are shown in Figs. 1 and 2. The spectroscopic data from FT-IR, FT-Raman, ^{1}H and ^{13}C NMR, do provide useful information for their formation and structural characterization. These data and their assignments are reported in Tables 1–4. Optimized structures of ligand and complexes are presented in Figs. 3–5. Required analytical data and physical properties are summarized in the end of each experimental.

3. Theoretical calculation

Optimizing a molecule involves finding the most stable geometry, or minimum energy structure, of a chemical sample. CACHE work system pro version 5.02 package program uses three different methods for geometry optimization of a molecule: Optimizing with mechanics, MOPAC or DGauss. The most widely used of these methods are the MOPAC and DDauss calculations methods. MOPAC parameters are obtained by fitting experimental heats of formation, geometries, permanent dipoles, and ionization potentials, whereas DGauss uses density functional theory (DFT) methods. DFT models provide an approximate solution to the Schrödinger equation using only mathematical approximations. There are many different sets of calculation for each of these methods. We have examined several of these methods, such as on MOPAC (AM1, MP3, MP5, MNDOd), and on DGauss (B88-PW91, B88-LYP and D-VWN all using DFT methods). We found that the B88-PW91 set gives the most stable structure for both the ligand and the complex.

Table 1
Some prominent IR bands of (L) and $M(L)X_2$ complexes at 3350–170 cm^{-1} region

Ligand (L)	1930, 1890, 1772 ν(ar. patterns), 1625 ν(C=C), 1590 ν_{as}(C=N), 1440 ν_{a}(C=N), 1273 ν(C–N), 735 δ(C=C_{ar}), 506, 477, 438, 361, 307, 270, 218
$Zn(L)Cl_2$	3325 ν(N–H), 1949, 1910, 1791 ν(ar. patterns), 1623 ν(C=C), 1596 ν_{as}(C=N), 1452 ν_{s}(C=N), 1155 ν(C–N), 748 δ(C=C_{ar}), 432 δ(C=H_{ar}), 514, 486 ν(Zn–N), 438, 363, 320-248 (v. br.) ν_{as}(Zn–Cl), 197 ν_{s}(Zn–Cl)
$Zn(L)Br_2$	3325 ν(N–H), 1623 ν(C=C), 1595 ν_{as}(C=N), 1451 ν_{s}(C=N), 1279 ν(C–N), 748 δ(C=C_{ar}); 512, 487 ν_{as}(Zn–N), 438 δ(C=H_{ar}), 253 ν_{as}(Zn–Br), 198 ν_{s}(Zn–Br)
$Zn(L)I_2$	3329 ν(N–H), 1623 ν(C=C), 1595 ν_{as}(C=N), 1448 ν_{s}(C=N), 1278 ν(C–N), 750 δ(C=C_{ar}), 512, 487 ν_{as}(Zn–N), 437 δ(C=H_{ar}), 359, 303, 288, 250 ν_{s}(Zn–N), 209, 201, 183ν_{as}(Zn–I)

Table 2
Prominent Raman bands of (L), metal halides and complexes at 3350–100 cm^{-1} region (solid)

$ZnCl_2$ ν(228), $ZnBr_2$ ν(164), ZnI_2 ν(121) and $PdCl_2$ ν(279)	
Ligand (L)	3070, 3060 ν(C–H_{ar}), 2975 ν(C–H), 2932 ν(C–H), 1626 ν(C=N), 1595 ν(C=C_{ar}), 740 δ(C=C_{ar}), 578, 516, 439, 360, 308, 279, 151
$Zn(L)Cl_2$	3082 ν(C–H_{ar}), 2942, 2926, 2894 ν(CH_2), 1600 ν(C=C), 1537 ν(C=N), 1450 ν(C=N), 1280 ν(C–N), 744 δ(C=C_{ar}), 488 ν_{as}(Zn–N), 252 ν_{s}(Zn–N), 251 ν_{as}(Zn–Cl), 196 ν_{s}(Zn–Cl)
$Zn(L)Br_2$	3074, 3063 ν(C–H_{ar}), 2968, 2942, 2927 ν(CH_2), 1621 ν(C=C), 1595 ν_{as}(C=N), 1529, 1451 ν(C=N), 1281, 1252 ν(C–N), 760 δ(C=C_{ar}), 488 ν_{as}(Zn–N), 365, 288, 251, ν_{s}(Zn–N), 251 ν_{s}(Zn–N), 180 ν_{as}(Zn–Br), 143 ν_{s}(Zn–Br)
$Zn(L)I_2$	3067 ν(C–H_{ar}), 2969, 2944 ν(CH_2), 1622 ν(C=N), 1595 ν_{as}(C=C), 1448 ν(C=N), 1278 ν(C–N), 750 δ(C=C_{ar}), 488 ν_{as}(Zn–N), 251ν_{s}(Zn–N), 251 ν_{s}(Zn–N), 180 ν_{as}(Zn–I), 143 ν_{s}(Zn–I)

4. Pharmacology

Fresh clinical isolates are obtained from Faculty of Medicine, University of Uludag (Turkey), and are supplemented by stock cultures of fresh clinical isolates of several species to achieve target numbers. The total numbers of isolates are

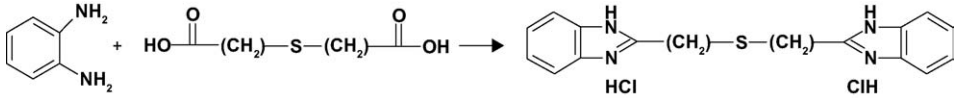

Fig. 1. Syntheses of ligand (L) + 2HCl, refluxed in 5 M HCl for 24 h.

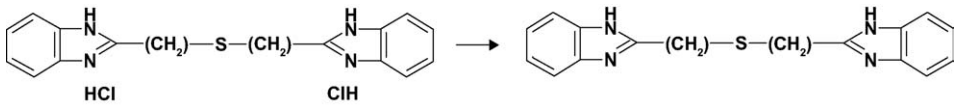

Fig. 2. Syntheses of ligand (L) neutralized with dilute base.

Table 3
^{1}H NMR spectra data of (L) and M(L)X_2 complexes (DMSO-d6)

Ligand (L)	δ_H 3.85 (4H, s, CH_2), 6.96 (4H, s, H_5 and H_6), 7.32 (4H, s, H_4 and H_7), (2H, NH not detected)
Zn(L)Cl_2	δ_H 4.06 (4H, s, CH_2), 7.39 (4H, s, H_5 and H_6), 7.68 (2H, s, H_7), 8.29 (2H, s, H_4), 13.68 (2H, s, NH)
Zn(L)Br_2	δ_H 4.02 (4H, s, CH_2), 7.40 (4H, s, H_5 and H_6), 7.70 (2H, s, H_7), 8.28 (2H, s, H_4), 13.68 (2H, s, NH)
Zn(L)I_2	δ_H 4.10 (4H, s, CH_2), 7.3.95 (4H, s, H_5 and H_6), 7.83 (4H, s, H_4 and H_7), 13.80 (2H, br, NH)
Pd(L)Cl_2	δ_H 4.56 (2H, d, $J_{Hgg'}$ = 15.20 Hz), 5.55 (2H, d, $J_{Hg'g}$ = 15.20 Hz), 7.2–7.6 (6H, m, H_5, H_6 and H_7), 8.60 (2H, d, H_4 : J = 8.00), 13.38 (2H, s, NH)

Table 4
$^{13}C\{^1H\}$ NMR (125 MHz) spectra data of (L) and M(L)X_2 complexes (DMSO-d_6)

Ligand (L)	δ_C 29.04 (2C, CH_2), 115.17 (4C, C_4 and C_7), 122.19 (4C, C_5 and C_6), 138.93 (4C, C_8 and C_9), 152.10 (2C, C_2)
Zn(L)Cl_2	δ_C 25.17 (2C, CH_2), 112.25 (2C, C_7), 118.05 (2C, C_4), 123.85 (2C, C_6), 124.25 (2C, C_5), 132.36 (2C, C_8), 139.10, (2C, C_9), 151.95 (2C, C_2)
Zn(L)Br_2	δ_C 25.11 (2C, CH_2), 112.30 (2C, C_7), 118.10 (2C, C_4), δ 123.65 (2C, C_6), 124.15 (2C, C_5), 132.85 (2C, C_8), 139.0, (2C, C_9), 151.80 (2C, C_2)
Zn(L)I_2	25.10 (2C, CH_2), 12.45 (2C, C_7), 118.25 (2C, C_4), 123.75 (2C, C_6), 124.18 (2C, C_5), 132.93 (2C, C_8), 139.20, (2C, C_9), 151.78 (2C, C_2)
Pd(L)Cl_2	δ_C 32.17 (2C, CH_2), 112.83 (2C, C_7), 118.35 (2C, C_4), 123.72 (2C, C_6), 124.44 (2C, C_5), 132.66 (2C, C_8), 139.98, (2C, C_9), 153.92 (2C, C_2)

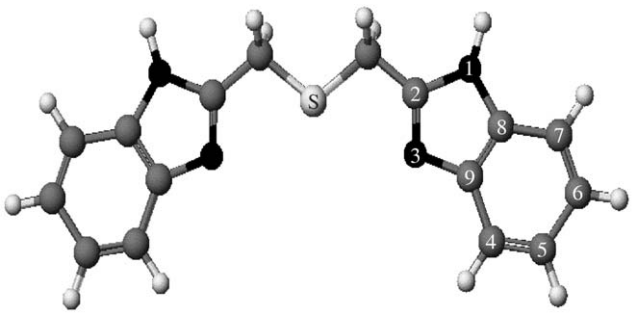

Fig. 3. Optimized structure of ligand (L).

115 bacteria and 30 fungi, representing 10 and three species, respectively (Tables 5 and 6).

Standardized powder samples of ampicillin (aminopenicillin, broad-spectrum antibiotic for treatment of Gram-negative and Gram-positive bacteria), ciprofloxacin (Cipro is considered the "gold standard" therapy for many types of Gram-negative infections, including *Pseudomonas aeruginosa*, and has approved for the inhaled form of anthrax), cefazolin (a semisynthetic cephalosporin antibiotic for intramuscular or intravenous administration), floxacin (broad activity anti-infective drug used for the treatment of lower respiratory infections and with inhibition of DNA-gyrase activity), piperacillin (β-lactamase a sensitive antibiotic effective on Gram-negative and Gram-positive bacteria), are purchased

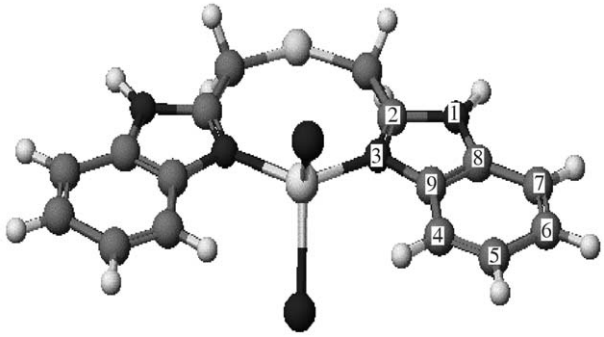

Fig. 4. Optimized structure of Zn(L)X_2 complex.

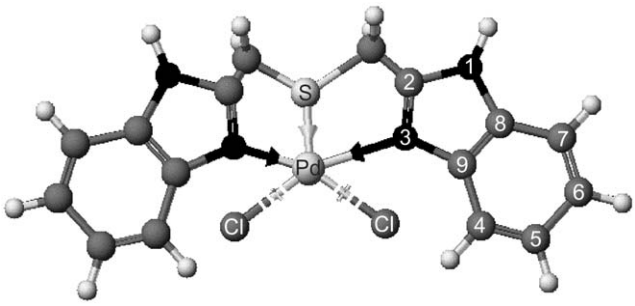

Fig. 5. Optimized structure of Pd(L)Cl_2 complex.

together with NAD from Sigma Chemicals. Amphotericin-B (antifungal and antiprotozoal commonly used for treatment of mycotic infection, including cryptococcosis, histoplasmosis, blastomycosis, candida, and also used medication for HIV and AIDS patients), fluconazole (an azole antifungal agent for treatment of oral thrush and vaginal infections), and flucytosine (for treatment of susceptible fungal infections, such as *Cryptococcus*) are obtained from Fluka. Mueller Hinton media, nutrient broth and malt extract broth are purchased from Difco and yeast extracts is obtained from Oxoid.

5. Results and discussion

5.1. Chemistry

5.1.1. Infrared and Raman spectra

The N–H stretching of imine group, which is not detected in the free ligand due to the fluxional behavior of the imine hydrogen atoms, appears in all four complexes as a medium broad band at ca. 3325 cm^{-1}. This supports the contention that coordination most probably occurs through both bis-3 and 3′ nitrogen atoms, causing inhibition of the fluxional behavior of imine hydrogen atoms.

The characteristic ν(CH) modes of ring residues and ethylenic group are observed as expected in the 3100–2850 cm^{-1} spectral regions in both IR and Raman for the ligand and the complexes. Specific differences between spectra in this region show strong support for the formation of new complexes (Tables 1 and 2). Slight down field shift of ν_{as}(C=N) vibrations, and appearance of strong bands at ca. 1500 cm^{-1} for

Table 5
Antibacterial activities of the ligand (L) and complexes, evaluated by MIC for 10 bacteria and their rate of susceptibility to five compared antibiotics

M	N	MIC (μg ml^{-1})[a]															% isolates MICs < 64 μg ml^{-1}					% isolates suspectible[b]				
		(L)			$Zn(L)Cl_2$			$Zn(L)Br_2$			$Zn(L)I_2$			$Pd(L)Cl_2$												
		50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	**1**	**2**	**3**	**4**	**5**	AM	CI	CF	OF	PI
A	15	32	64	32–128	32	32	32–128	32	64	32–128	32	128	32–128	8	16	8–32	85	90	96	88	94	7	60	70	50	60
B	15	16	32	16–32	16	16	8–16	8	16	8–16	8	16	8–16	8	16	8–16	100	100	100	100	100	67	97	93	100	67
C	15	16	16	16–32	8	16	8–16	8	32	8–32	8	16	8–16	8	32	8–32	100	100	100	100	100	0	100	6	100	88
D	10	8	16	8–32	4	8	4–8	8	8	4–8	4	8	4–8	8	8	4–8	100	100	100	100	100	12	100	96	100	88
E	10	16	16	16–32	8	8	4–8	8	16	8–16	8	16	8–16	8	16	8–16	100	100	100	100	100	100	80	0	80	100
F	10	16	32	16–32	8	16	8–16	8	16	8–16	8	16	8–16	8	16	4–16	100	100	100	100	100	16	100	4	92	96
G	10	32	64	32–128	32	64	32–128	32	128	32–128	32	64	32–128	16	16	16–32	92	97	96	96	94	100	100	100	97	100
H	10	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	0	0	0	0	0	100	96	85	92	100
I	10	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	0	0	0	0	0	100	96	85	94	100
J	10	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	> 256	> 256	256–> 256	0	0	0	0	0	100	87	0	83	100

M: microorganimsms; N: number of isolates. (A) *S. aureus*, (B) *E. coli*, (C) *E. aerogenes*, (D) *K. pneumoniae*, (E) *A. faecalis,* (F) *C. freundii*, (G) *S. pyogenes*, (H) *P. vulgaris*, (I) *P. mirabilis*, (J) *E. faecalis*. **1** = Ligand (L), **2** = $Zn(L)Cl_2$, **3** = $Zn(L)Br_2$, **4** = $Zn(L)I_2$, **5** = $Pd(L)Cl_2$.

[a] 50% and 90%, MICs at which 50 and 90% of the isolates are inhibited, respectively.

[b] Susceptibility breakpoints are in parentheses with μg ml^{-1} values. AM, ampicillin (< 8.0); CI, ciprofloxacin (< 1.0); CF, cefazolin (< 8.0); OF, ofloxacin (< 2.0); PI, piperacillin (< 16).

Table 6
Antifungal activities of the ligand (L) and complexes, evaluated by MIC for three yeasts and their rate of susceptibility to five compared antifungal

M	N	MIC (μg ml^{-1})[a]															% isolate, MICs < 64 μg ml^{-1}					% isolate sus.[b]		
		(L)			$Zn(L)Cl_2$			$Zn(L)Br_2$			$Zn(L)I_2$			$Pd(L)Cl_2$										
		50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	**1**	**2**	**3**	**4**	**5**	AM	FLU	FL
A	10	8	8	8–16	4	8	4–8	4	8	4–8	2	4	≤ 0.125-4	4	8	4–8	100	100	100	100	100	100	100	100
B	10	8	8	2–8	4	8	4–8	4	8	4–8	2	4	≤ 0.125-4	4	8	4–8	100	100	100	100	100	100	100	100
C	10	8	8	8–16	4	8	4–8	4	8	4–8	4	8	4–8	4	8	4–8	100	100	100	100	100	98	100	92

M: microorganisms; N: number of isolates. (A) *C. albicans*, (B) *C. utilis*, (C) *C. neoformans*. **1** = Ligand (L), **2** = $Zn(L)Cl_2$, **3** = $Zn(L)Br_2$, **4** = $Zn(L)I_2$, **5** = $Pd(L)Cl_2$.

[a] 50% and 90%, MICs at which 50% and 90% of the isolates are inhibited, respectively.

[b] Susceptibility breakpoints are in parentheses with μg ml^{-1} values. AM, amphotericin-B (< 16); FLU, fluconazole (< 8.0); FL, flucytosine (< 16.0).

the *v*s C=N stretch, in IR and Raman tends to support prohibition of imine delocalization [17,18]. These absorptions support the argument that coordination most probably occurs through imine nitrogen atoms.

Complex formation could also be confirmed particularly in the lower frequency region, due to the changes in ν(M–N) and ν(M–X) vibrational modes. The anhydrous metallic halides ZnX_2 (X = Cl, Br, and I) and $PdCl_2$ show strong stretching vibrations in the Raman spectra. These bands changed significantly due to complexation (Table 2). Furthermore, the metal-nitrogen vibrations are expected to be similar in spectral frequency and quite weak, due to the aromatic and planar characteristic of coordination section of the ligand. These vibrational absorptions are assigned in Tables 1 and 2.

5.1.2. Nuclear magnetic resonance

The ^{1}H NMR spectra pattern changes significantly in the complexes due to the coordination. The unresolved imine proton atoms for the free ligand are observed as singlets at ca. δ 13 ppm for all complexes. This strongly supports that coordination most probably occurs *via* both bis-3 and 3′ nitrogen atoms, causing inhibition of the fluxional behavior of imidazole ring, which was also indicated by vibrational spectra.

In the Zn complexes there are small (about 0.2 ppm) downfield shifts occur for CH_2 protons, which may cause due formation of large cyclic molecule and at the same time by the position of halogen atoms on these particular protons, which may also caused this small de-shielding effect. If the sulfur atom is coordinated to the metal ion, it should have caused significant changes on bridging CH_2 protons due to the formation of bicyclo[4.4.0] octane type complex. The formation of geminal proton atoms, will exhibit two sharp doublets with a big coupling constants, as observed for $Pd(L)Cl_2$ complex, which we have reported previously (Table 3) [19].

The ^{13}C NMR spectrum of the ligand exhibits five signals. Upon complexation, the fluxional behavior of the imine proton is inhibited and the complexes exhibit eight signals. The CH_2 resonance shifts from about δ 29 to 25 (average 4 ppm) for zinc complexes. This upfield resonance on the carbon atom, in spite on downfield resonance on the proton atoms, probably is caused by large cyclic formation, which would have weakened effect on the formation of intermolecular hydrogen bonds, particularly on the sulfur atom. This shielding tends to support coordination *via* the nitrogen atoms instead of sulfur. The sulfur atom coordination will result in a strong de-shielding effect on this particular carbon atom, causing downfield resonances instead of upfield, which is observed in the case of palladium complexes [19]. The NMR spectra data and their assignments are presented in Tables 3 and 4.

5.2. Microbial activity

The results concerning in vitro antimicrobial activities of the ligand and the complexes together with the minimal inhibitory concentration (MIC) values of compared antibiotic and antifungal are presented in Tables 5 and 6, respectively. The total numbers of isolates are 115 pathogenic bacteria and 30 yeasts, representing 10 and three species, respectively. The data obtained for these compounds are compared with those of known antibacterial agents, ofloxacin, ciprofloxacin, piperacillin, ampicillin, cefazolin and as well as effective antifungals, namely, amphotericin, fluconazole and flucytosine. Data from Table 5 indicate that, apart from some exceptions, all the tested compounds exhibit equipotent or strong antibacterial activity than those referenced antibiotics. Exception arise towards microorganisms, namely, *Proteus vulgaris*, *Proteus mirabilis*, and *Enterococcus faecalis*, which either the ligand and complexes have no effect at all and requires over 64 μg ml^{-1} doses for their inhibition. But most of the compared antibiotics, particularly ampillicin and piperacillin show very strong activity on these organisms. The MIC values in Table 6 indicate that all the tested compounds exhibit a very strong antifungal and penetrating activity on all three species.

The inhibition activity seems to be governed in certain degree by the facility of coordination at the metal center, because the complexes are the most active compared to the free ligand. The zinc tetrahedral and palladium square pyramidal complexes show similar activities towards all tested organisms. This tends to support that, the type of coordination and as well as the structural differences, do not play an important role on their biological activity.

It is known that zinc metalloenzyme phosphomannose isomerase (PMI) plays an essential role in organisms cell wall biosynthesis and that this enzyme is inhibited by silver sulfadiazine, clinically used as a topical antibacterial and antifungal agent, through binding of metal center with the cysteine in proteins [20]. Taking the high affinity of the zinc and palladium center with nitrogen atoms (in proteins and nucleobases) into account, the mechanism of action of the metal center is plausibly similar to that of the silver agent. This supports the argument that, apart from electrostatic attraction from the ligand side, some type of coordination may be occurring between the organism and the metal center, causing the inhibition of biological synthesis and preventing the organisms from reproducing. Due to their structural differences, binding mechanism of the Pd(II) and Zn(II) complexes to the organisms also could be very different. For instant, the rare palladium five coordinate complex may cleave one or even two chloride ions upon contact with the organisms, forming five-coordinate (1:1 electrolyte) and four-coordinate (2:2 electrolyte square planar) ionic species, respectively. But, in the case of Zn(II) a part from four-coordinate ionic complexes due to the halide dissociation, formation of five coordinate on the metal center upon contact with organism, is also a possibility. These coordination forces may be indicative of the effectiveness of the complexes on most organisms compared with free ligand. Similarly, the referenced ampicillin and ofloxacin antibiotics are thought to exert their antimicrobial effect by entering the bacterial cell and inhibiting DNA-gyrase, which is involved in the production of genetic material, preventing the bacteria from reproducing.

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 [21]. However, in this study, the compounds are active against both Gram-positive and Gram-negative bacteria and highly active against yeasts. The activity against both types of bacteria and yeasts may be indicative of the presence of broad-spectrum.

The results of our study indicate that the compounds have the potential to generate novel metabolites, by displaying high affinities for most of the receptors. Their strong effect on many tested organisms, particularly their lethal anticandidal activity could result in the discovery of novel anticandidal agents, demonstrating broad-spectrum characteristic. These complexes could be selected for further pharmacological tests to be evaluated as potential drugs against many infectious diseases.

6. Experimental protocols

6.1. Chemistry

All chemicals and solvents are reagent grade and are used without further purification. Purity of the compounds is tested on thin layer chromatography as well as vibrational spectroscopy. Melting points are determined with Electrothermal 9100 melting point apparatus. Analytical data are obtained with Carlo Erba 1106 analyzer. FT-IR spectra are recorded (mid-IR as KBr pellets and far-IR in polyethylene tablets) on a Jasco FT/IR-600 Plus Spectrometer. FT-Raman spectra are obtained from powdered samples placed in a Pyrex tube using the Bruker RFS 100/S spectrometer (Nd:YAG-Laser, 1064 nm, 200 mW). Raman and IR peaks are labeled ν, stretching; ν_{as}, anti-symmetric stretching; ν_s, symmetric stretching; δ, bending. Routine ^1H and ^{13}C NMR spectra are recorded at ambient temperature; on a 500 MHz NMR Spectrometer in deuterated dimethyl sulfoxide (DMSO-d_6). Chemical shifts (δ) are expressed in units of ppm relative to TMS. Coupling constants (J) are given in Hz and NMR peaks labeled as singlet (s), doublet (d), triplet (t), multiplet (m) and broad (b).

6.1.1. Synthesis

6.1.1.1. The 1,3-bis(2-benzimidazyl)-2-thiapropane Ligand (L). The 1,3-bis(2-benzimidazyl)-2-thiapropane ligand (L) was prepared according to literature methods [9,19], by using a mixture of 2,2′-thiodiethanoic acid (2.0 g, 13.3 mmol) and freshly sublimed *o*-phenylenediamine (3.63 g, 26.7 mmol) in 5 M HCl (30 ml) at reflux temperature. The cooled precipitates were collected and identified as (L)·2HCl (4.90 g, 80%). The free base ligand (L) was obtained by treatment of (L)·2HCl with an aqueous 10% ammonia (3.30 g, 83%, m.p. 221 °C).

6.1.1.2. Zn (L) Cl_2 complex. A solution mixture of 1,3-bis(2-benzimidazyl)-2-thiapropane(L) (100 mg, 0.34 mmol) and $ZnCl_2$ (48 mg, 0.35 mmol) in absolute EtOH (6 cm^3) is refluxed overnight. The white solid is collected, and washed several times with small portions of ethanol to render it pure (113 mg, 77%). Dec. > 300 °C. Found (calculated): C, 44.2 (44.7); H, 3.5 (3.3); N, 12.8(N, 13.0%); $C_{16}H_{14}Cl_2N_4SZn$.

6.1.1.3. Zn(L)Br_2 complex. This white crystalline complex is prepared in a manner similar to that used for Zn(L)Cl_2 using a mixture of (L) (100 mg, 0.34 mmol) and $ZnBr_2$ (78 mg, 0.35 mmol) in absolute EtOH (5 ml). The yield was (120 mg, 68%). Dec. > 300 °C. Found (calculated): C, 36.8 (36.9); H, 2.9 (2.7); N, 10.7 (10.8%); $C_{16}H_{14}Br_2N_4SZn$.

6.1.1.4. Zn(L)I_2 complex. This white solid complex was prepared in a manner similar to that used for Zn(L)Cl_2 reacting a mixture of (L) (100 mg, 0.34 mmol) and ZnI_2 (110 mg, 0.35 mmol) in absolute EtOH (6 ml). The yield was (132 mg, 63%). Dec. > 300 °C. Found (calculated): C, 30.9 (31.3); H, 2.5 (2.3); N, 8.8 (9.1%); $C_{16}H_{14}I_2N_4SZn$.

6.1.1.5. Pd(L)Cl_2 complex [19]. Deoxygenated solutions of (L) (70 mg, 24 mmol) and $PdCl_2$ (42 mg, 24 mmol) in absolute EtOH (6 cm^3) are refluxed under a N_2 atmosphere with constant stirring overnight. The mixture is cooled and the light yellow solid product is collected (90 mg, 85%). M.p. > over 350 °C.

6.2. Pharmacology

6.2.1. Microorganisms

The antimicrobial activities are evaluated against Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*, *E. faecalis*) and Gram-negative (*Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Alcaligenes faecalis*, *Citrobacter freundii*, *P. vulgaris*, *Proteus mirabilis*) bacteria and the yeast cultures *Candida albicans*, *Candida utilis*, *Cryptococcus neoformans*.

6.2.2. Antimicrobial screening

Screening for antibacterial and antifungal activities are carried out by preparing a broth micro dilution, following the procedure outlined by the National Committee for Clinical Laboratory Standards - NCCLS [22].

All the bacteria are incubated and activate at 30 ± 0.1 °C for 24 h by inoculation into nutrient broth (Difco), and the yeasts are incubated in malt extract broth (Difco) for 48 h. A cation adjusted Mueller–Hinton broth is used for testing the susceptibility of microorganisms to antimicrobial agents. For streptococci and enterococci, the medium is supplemented with 5% lyzed horse blood, 5 mg ml^{-1} of yeast extract, and 15 μg ml^{-1} of NAD. The final inoculation (inoculums) approximately was 10^5 cfu ml^{-1}. For 50 of the isolates, bactericidal endpoints are determined, following the method recommended by the NCCLS [23]. Susceptibilities of fungi are performed by the broth macro dilution testing method using RPMI 1640 broth [24]. The compounds are dissolved in dim-

ethyl sulfoxide (DMSO). To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO as same procedures as used in the experiments. Tested concentrations of the compounds are serial twofold dilutions ranging from 256 to 0.125 μg ml^{-1}. The control tubes are incubated at 35 °C (24 h) and at 25 °C (72 h) for bacteria and yeasts, respectively. The MIC determined at the end of incubation period. The lowest concentrations of antimicrobial agents that result in complete inhibition of microorganisms are represented as MIC μg ml^{-1} in Tables 5 and 6.

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