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Synthesis, characterisation and antifungal activity of a series of Cobalt(II) and Nickel(II) complexes with ligands derived from reduced *N, N'*-*o*-Phenylenebis(Salicylideneimine)

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Abstract The synthesis and characterisation by elemental analysis, conductivity, FTIR, UV–Visible, ESR and magnetic measurements are described for a series of complexes of nickel(II) and cobalt(II) with three ligands (H_2L^{1-3}) derived from reduced *N, N'*-*o*-Phenylenebis(salicylideneimine). The complexes formed are identified as neutral species, where the ligands are coordinated through N and O donor atoms. The formulae obtained for the complexes are: $[CoL(H_2O)_2]$ with octahedral geometry and $[NiL]$ with tetrahedral geometry. Their antifungal activity is evaluated towards human pathogenic fungi including yeasts of the *Candida* genus, some opportunistic moulds belonging to the *Aspergillus*, *Scedosporium* genus and some dermatophytes. The cobalt complexes show a significant growth inhibition of yeasts tested and also to fungi of the genus *Scedosporium* which is of interest because these fungi are usually poorly susceptible to current antifungal including Amphotericin B and Itraconazole, chosen as reference in this study. The activity data show that the metal complexes are more potent than the parent ligand.

Introduction

The Schiff bases have been subject of great of interest for a number of years because of their various chemical and structural characteristics, and also their proved applications as biologically active molecules [1]. Their complexes are known to be biologically important and act as models to understand the structure of biomolecules and metalloproteins. They have also a variety of applications, including biological, clinical, analytical and also industrial purposes [2].

With increasing incidence of deep mycosis in recent years [3], there were more and more studies for screening new and more effective antimicrobial broad-spectrum drugs with low toxicity. The interest in the study of Schiff bases and their complexes containing oxygen and nitrogen donor atoms arises from their significant antifungal activity [4–6]. Inhibiting properties toward tumour growth were recently demonstrated for some Schiff base complexes [7].

The imine bond in Schiff bases can be easily reduced to give amino derivatives. The comparison of the ligand coordinating properties of reduced Schiff bases with their Schiff base parents showed that the basicity of N atoms is enhanced and also a greater flexibility as consequence of the hydrogenation of the C=N bond, leading to more stable complexes [8–11]. For these reasons, the reduced Schiff bases have gained particular attention [12, 13].

We have previously reported [6] a synthesis and characterisation of manganese(II) and copper(II) complexes with reduced Schiff bases, and their antifungal activity towards some human pathogenic fungi. The manganese complexes showed a significant growth inhibition of the dermatophytes tested and the fungi of the genus *Scedosporium*. This is very interesting as these fungi are usually poorly susceptible to current antifungals.

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To pursue these studies, we have chosen cobalt(II) and nickel(II) complexes. We report here, their synthesis, characterisation by elemental analysis, conductimetry, FTIR, UV–Visible, ESR and magnetic measurements and the results of their antifungal activities towards human pathogenic fungi.

Experimental

Reactants

All reactants and solvents used were analytical grade. The ligands were synthesised as previously described [11]. Hexahydrated cobalt and nickel chloride were purchased from Merck (France) and used without further treatment.

Measurements

Elemental analyses were carried out by the service d'Analyse, ICSN–CNRS, Gif sur Yvette (France). Melting points were determined with a digital melting point apparatus using capillary technique. The molar conductance measurements were carried out at 25 °C using a Tacussel CD810 conductivity meter on 10^{-3} M solutions in DMSO.

IR spectra were obtained using a Perkin Elmer FT-IR spectrometer Spectrum-one Model, between 400 and 4,000 cm^{-1} (KBr disks). Electronic spectra were recorded with a Shimadzu UV 1601 spectrophotometer in DMSO solutions. The ESR measurements were performed in solution and in solid state, at both room temperature and 113 K, on a Bruker EMX spectrophotometer (Université du Maine, France) and theoretical spectra were calculated with the help of SimFonia Bruker software. This programme uses a set of perturbation theory algorithms to quickly explore the parameter spaces involved in powder and solution spectra.

The magnetic measurements were carried out using a SQUID magnetometer in the 200–300 K range with a 10,000 G applied field, diamagnetic corrections were estimated from Pascal's constants and magnetic data were corrected for diamagnetic contributions of the sample holder.

Preparation of the complexes

The complexes were prepared by refluxing for 4–5 h, 1 mmol of the hexahydrated metal chloride with 1 mmol of the ligand in absolute EtOH. The complexes precipitated as powders which were removed by filtration, washed with water–ethanol mixture (50/50, v/v), and finally dried in vacuum.

Biological activity of complexes

Microorganisms and culture conditions

Antifungal activity was assayed on different human pathogenic fungi, including yeasts of the *Candida* genus (*C. albicans* ATCC 66396, *C. glabrata* LMA 90-1085, *C. tropicalis* LMA 91-4959 and *C. parapsilopsis* LMA 93-8558), some opportunistic moulds belonging to the *Aspergillus* (*A. fumigatus* CBS 11326, *A. terreus* LMA 40700121, and *A. flavus* LMA 40110521) or *Scedosporium* genus (*S. apiospermum* LMA 90-4595 and *S. prolificans* LMA 40409298), and some dermatophytes (*Microsporum gypseum* 50500961, *Microsporum persicolor* LMA 92-806, *Trichophyton mentagrophytes* LMA 90-406 and *Trichophyton tonsurans* LMA 50600608). Yeasts and moulds were cultivated at 37 °C on yeast extract–peptone–dextrose agar (YPDA) containing 0.5 g/L chloramphenicol for 2 or 3 days for yeasts and opportunistic moulds, respectively. As for the dermatophytes, they were cultivated at 25 °C during 7 days on YPDA plates containing chloramphenicol and supplemented with 0.5 g/L cycloheximide (Sigma) to avoid contamination by susceptible moulds like the aspergilli. After incubation, the inoculum suspension was prepared for yeasts by suspending one colony in 10 mL of sterile distilled water. For filamentous fungi, the mycelium was recovered by scraping the agar plates with 10 mL of sterile distilled water. For the sporulating aspergilli, conidia were then harvested by filtration through 25- μm pore size nylon Monyl membranes and finally, absorbance of the filtrate at 630 nm was adjusted spectrophotometry to 0.1. As for *Scedosporium* spp. and the dermatophytes, fungal suspensions were homogenised with a ground-glass grinder and finally adjusted by spectrophotometry to an A_{450} of 0.4.

Determination of antifungal activity

Antifungal activity of the different compounds (ligands, metal salts and complexes) was evaluated using a disk diffusion method routinely used for yeasts [14], and adapted here for dermatophytes and opportunistic moulds. Briefly, compounds were dissolved in DMSO at a final concentration of 10 mg/mL and 25- μL aliquots were applied on 12-mm diameter paper disks (rf 06234304, Prolabo 33173 Gradigan, France). After evaporating the solvent, disks were deposited in the centre of 90-mm diameter Casitone agar plates previously flooded with 10 mL of spore or hyphal fragment suspensions. Negative control was performed using filter papers soaked with an equal volume of respective metal salts and drug-free solvent (DMSO), and positive controls were made with Neosensitabs tablets of Amphotericin B or Itraconazole (Rosco Diagnostic, Taastrup, Denmark). After incubation,

diameter of the growth inhibition zones (mm) was measured around the paper disks or Neosensitabs tablets.

Results and discussion

The physical properties and analytical data of the complexes are summarised in Table 1. The nickel and cobalt complexes were obtained as powders and attempts to obtain single crystal suitable for X-ray determination were unsuccessful. They are insoluble in water, partially soluble in methanol and ethanol, totally soluble in DMF and DMSO.

The elemental analysis and all the physical measurements permit the suggestion of these empirical formula: $[\text{CoL}(\text{H}_2\text{O})_2]$ and $[\text{NiL}]$. The molar conductivities of the complexes in DMSO indicate that they are non-electrolyte species [15].

IR spectra

These ligands are expected to act as tetradentate ones through nitrogen and oxygen atoms, as confirmed by X-ray structures reported for similar ligands [16–18].

A study and a comparison of the IR spectra of our ligands and their cobalt(II) and nickel(II) complexes allow us to determine the coordination sites of the ligands. The IR data is presented in Table 2.

The free ligands show an absorption band near $3,200\text{ cm}^{-1}$ assigned to NH stretching vibration. A strong band is observed in the $1,500\text{--}1,600\text{ cm}^{-1}$ region assigned to NH deformation and C=C stretching vibration. The C–N and C–O stretching vibrations bands are observed at $1,250$ and $1,150\text{ cm}^{-1}$, respectively, the vibration band of phenolic OH is observed at ca. $3,050\text{ cm}^{-1}$ [19].

The most important changes occurring after complexation are:

- The disappearance of the $\nu(\text{OH})$ band, due to the deprotonation of the ligands, leading to neutral complexes as indicated by conductimetric measurements.
- The shift to lower or higher wavenumbers of C–O and C–N vibration bands because of the involvement of oxygen and nitrogen atoms in the coordination. This is confirmed by the appearance of new bands around 400 and 500 cm^{-1} assigned to M–O and M–N bonds after complexation [20].

Finally, a broad band is observed around $3,400\text{ cm}^{-1}$ in the spectra of the cobalt complexes, assigned to coordinated water molecules.

Electronic spectra and magnetic measurements

The free ligands exhibit two intense bands in the $40,000\text{--}30,000\text{ cm}^{-1}$ region due to $\pi \rightarrow \pi^*$ transitions, which remain unchanged in the spectra of the complexes.

The electronic data of d–d transitions of the complexes are summarised in Table 2. The $[\text{CoL}^1(\text{H}_2\text{O})_2]$ complex exhibits a magnetic moment of 5.08 BM corresponding to high spin octahedral cobalt(II) species [21]. It is supported by its electronic spectra where we observe three absorption bands at $10,593$, $14,641$ and $21,505\text{ cm}^{-1}$ corresponding to: ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{2g}$, ${}^4\text{T}_{1g} \rightarrow {}^4\text{A}_{2g}$ and ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{1g}$ transitions, respectively [21–23].

The magnetic moments of the complexes $[\text{CoL}^2(\text{H}_2\text{O})_2]$ and $[\text{CoL}^3(\text{H}_2\text{O})_2]$ are 1.76 and 1.82 BM, respectively. They are typical of low spin octahedral cobalt(II) species. Their electronic spectra exhibit two bands around: $10,000$, and $20,000\text{ cm}^{-1}$, assigned to: ${}^2\text{E}_g \rightarrow {}^2\text{T}_{1g}$, ${}^2\text{T}_2$; ${}^2\text{E}_g \rightarrow {}^2\text{A}_{1g}$ transitions, respectively [21, 24, 25].

Table 1 Analytical data and magnetic moments of the complexes

Complexes	Colour	Elemental analysis found (calcd.) (%)			M.p. (°C)	Λ ($\Omega^{-1}\text{ cm}^2\text{ mol}^{-1}$)	μ_{eff} (BM)
		C	H	N			
$[\text{CoL}^1(\text{H}_2\text{O})_2]$	Dark green	(58.4)	(5.4)	(6.5)	205	32	5.08
		58.1	5.3	6.8			
$[\text{CoL}^2(\text{H}_2\text{O})_2]$	Brown	(59.6)	(5.3)	(7.0)	277	11	1.76
		59.0	5.6	6.6			
$[\text{CoL}^3(\text{H}_2\text{O})_2]$	Brown	(53.1)	(4.9)	(6.7)	256	15	1.82
		53.6	4.7	6.3			
$[\text{NiL}^1]$	Orange	(62.5)	(5.0)	(7.3)	242	18	2.32
		62.7	4.8	7.4			
$[\text{NiL}^2]$	Orange	(64.3)	(4.2)	(7.3)	261	24	2.56
		64.9	4.8	7.4			
$[\text{NiL}^3]$	Orange	(55.1)	(5.0)	(6.7)	257	21	2.39
		54.9	4.8	6.5			

Table 2 Relevant IR data (cm^{-1}) and electronic spectral data of the ligands and the complexes

Complexes	$\nu(\text{OH})^a$	$\nu(\text{OH})^b$	$\nu(\text{NH})$	$\nu(\text{C}-\text{O})$	$\nu(\text{C}-\text{N})$	$\nu(\text{C}=\text{O})$	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{O})$	ν^- (cm^{-1})	ϵ ($\text{L mol}^{-1} \text{cm}^{-1}$)	Electronic transitions
H_2L^1	–	3061 _m	3298 _s	1465 _s	1229 _m	1106 _m	–	–	30120 32573 36363	1750 5400 9850	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$
H_2L^2	–	3040 _m	3288 _s	1496 _s	1235 _m	1105 _m	–	–	30030 32362 36630	1800 6200 8750	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$
H_2L^3	–	3030 _m	3314 _s	1446 _s	1265 _m	1100 _m	–	–	27247 32362 37037	2200 6000 16500	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$
$[\text{CoL}^1(\text{H}_2\text{O})_2]$	3380 _{br}	–	3309 _m	1447 _m	1242 _m	1156 _m	521 _w 475 _w	418 _w 408 _w	10593 14641 21505	45 344 490	$^4\text{T}_{1g} \rightarrow ^4\text{T}_{2g}$ $^4\text{T}_{1g} \rightarrow ^4\text{A}_{2g}$ $^4\text{T}_{1g} \rightarrow ^4\text{T}_{1g}$
$[\text{CoL}^2(\text{H}_2\text{O})_2]$	3350 _{br}	–	3215 _m	1458 _m	1267 _m	1149 _m	500 _w 455 _w	433 _w 422 _w	10060 21052	18 852	$^2\text{E}_g \rightarrow ^2\text{T}_{1g}, ^2\text{T}_{2g}$ $^2\text{E}_g \rightarrow ^2\text{A}_{1g}$
$[\text{CoL}^3(\text{H}_2\text{O})_2]$	3375 _{br}	–	3204 _m	1460 _m	1250 _m	1149 _m	530 _w 468 _w	439 _w 416 _w	10131 21186	19 425	$^2\text{E}_g \rightarrow ^2\text{T}_{1g}, ^2\text{T}_{2g}$ $^2\text{E}_g \rightarrow ^2\text{A}_{1g}$
$[\text{NiL}^1]$	–	–	3223 _m	1466 _m	1264 _m	1158 _m	585 _w 523 _w	420 _w 408 _w	15037 17667 20833	157 210 446	$^3\text{T}_1 \rightarrow ^3\text{A}_2$ $^3\text{T}_1(^3\text{F}) \rightarrow ^3\text{T}_1(^3\text{P})$
$[\text{NiL}^2]$	–	–	3282 _m	1484 _m	1268 _m	1113 _m	545 _w 503 _w	453 _w 413 _w	15250 17667 21141	84 144 766	$^3\text{T}_1 \rightarrow ^3\text{A}_2$ $^3\text{T}_1(^3\text{F}) \rightarrow ^3\text{T}_1(^3\text{P})$
$[\text{NiL}^3]$	–	–	3260 _m	1448 _m	1266 _m	1116 _m	525 _w 492 _w	450 _w 416 _w	15400 17420 21052	110 209 760	$^3\text{T}_1 \rightarrow ^3\text{A}_2$ $^3\text{T}_1(^3\text{F}) \rightarrow ^3\text{T}_1(^3\text{P})$

^a Water molecules

^b Phenolic

br: broad; m: medium; s: strong; w: weak

All the nickel complexes were found to be paramagnetic species and show magnetic moments values in the 2.32–2.56 BM range, which are consistent with tetrahedral geometry. The coordination symmetry is probably C_{2v} [2, 26]. Their electronic spectra show three absorption bands at 15,000, 17,000 and 20,000 cm^{-1} [21]. The first can be assigned to $^3\text{T}_1 \rightarrow ^3\text{A}_2$ transition, those at 17,000 and 20,000 cm^{-1} can be attributed to transitions from $^3\text{T}_1(^3\text{F})$ to the terms arising by splitting of $^3\text{T}_1(^3\text{P})$ term in a crystal field of lower symmetry [21].

Electron spin resonance

The low spin cobalt complexes: $[\text{CoL}^2(\text{H}_2\text{O})_2]$ and $[\text{CoL}^3(\text{H}_2\text{O})_2]$ exhibit in the solid state at room temperature isotropic spectra. Their g_{iso} values are in the 2.003–2.008 range.

In DMSO solution and at 250 K, these complexes were inactive. When decreasing the temperature to 113 K

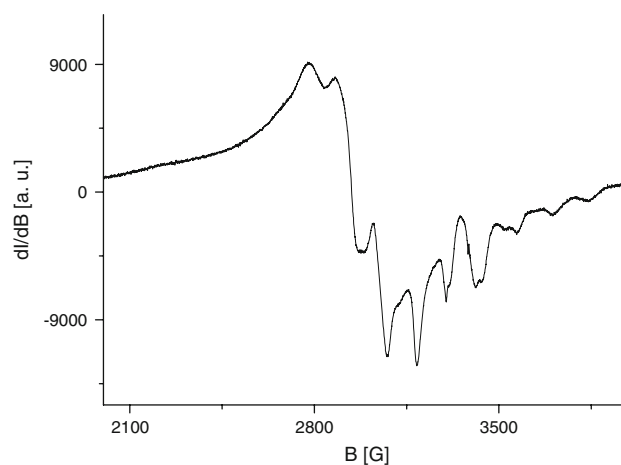


Fig. 1 ESR spectrum of $[\text{CoL}^3(\text{H}_2\text{O})_2]$ in DMSO solution at 113K

(vitreous state), hyperfine structure appears (Fig. 1) with eight hyperfine lines assigned to an interaction of an unpaired electron with ^{59}Co nucleus ($I = 7/2$).

The parameters obtained by simulation are:

For $[\text{CoL}^2(\text{H}_2\text{O})_2]$: $A_x = 113 \text{ G}$, $A_y = 70 \text{ G}$, $A_z = 150 \text{ G}$,
 $g_x = 2.28$, $g_y = 2.28$, $g_z = 2.05$.

For $[\text{CoL}^3(\text{H}_2\text{O})_2]$: $A_x = 120 \text{ G}$, $A_y = 40 \text{ G}$, $A_z = 140 \text{ G}$,
 $g_x = 2.28$, $g_y = 2.20$, $g_z = 2.02$.

These values indicate the absence of axial symmetry and suggest that the water molecules are in *cis* position.

The high spin $[\text{CoL}^1(\text{H}_2\text{O})_2]$ complex was inactive in both solid state and solution. This behaviour could be attributable to the very short spin-lattice relaxation time of the Co(II) ions in octahedral field, as previously reported for similar complexes [27, 28].

Complex structures

On the basis of these results, it appears that the ligands are coordinated in a deprotonated form, through oxygen and nitrogen atoms leading to neutral complexes. The cobalt complexes are found to be octahedral with two coordinated water molecules probably in *cis* position. The elemental analysis indicates that the nickel complexes are tetraordinated, their magnetic measurements suggest a tetrahedral geometry around nickel(II) ion. The proposed structures are given in Fig. 2.

Antifungal activity

The antifungal activity of the ligands and their complexes were tested *in vitro* against human pathogenic fungi, their activity was compared with those found for Amphotericin B and Itraconazole. The results are presented in Table 3.

As expected, no growth inhibition was observed for DMSO and metal salts. Amphotericin B and Itraconazole exhibited diameters ranging from 11 and 30 mm to 18 and 40 mm depending on the species, except for *S. prolificans* LMA 40409298 which was totally resistant to both drugs. In addition, Itraconazole was ineffective against *A. fumigatus* and *A. terreus*.

The results obtained as shown in Table 3, indicate that the cobalt complexes exhibited noticeable antifungal susceptibilities against all the tested moulds, especially *C. parapsilopsis*. On the opposite side, the nickel complexes have only a weak activity against *C. tropicalis* (ranging from 14 to 24 mm) and were quite inefficient against the others strains (data not shown).

The cobalt complexes showed a marked activity against yeasts and *Aspergillus* spp. with the largest effects attributed to the $[\text{CoL}^3(\text{H}_2\text{O})_2]$ complex. In this context because of their lack of activity, the corresponding ligands are not involved in the antifungal activity, suggesting that complexation with cobalt is totally implicated in the activity.

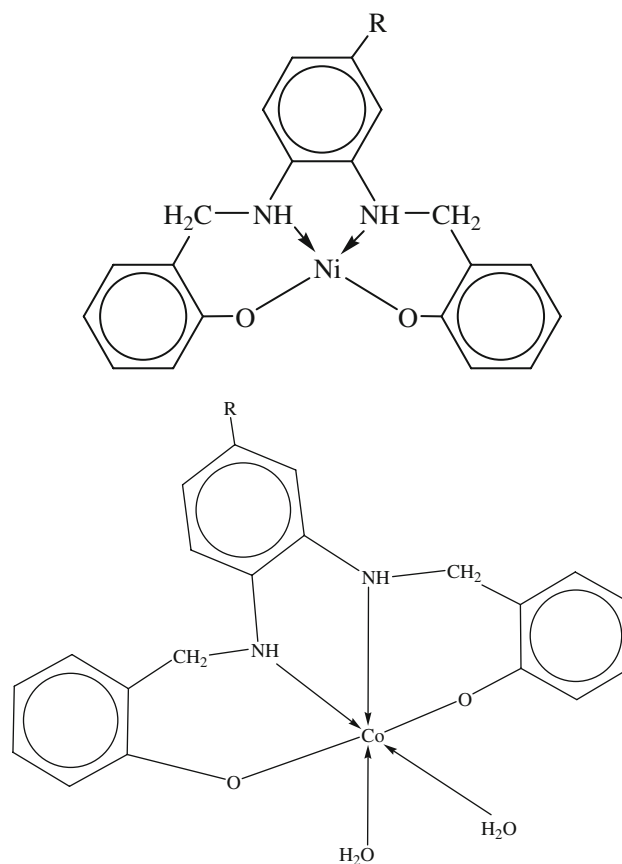


Fig. 2 Proposed structures for the complexes, R = H: H_2L^1 , R = CH_3 : H_2L^2 , R = Cl: H_2L^3

Strikingly, no growth inhibition was observed for the dermatophytes tested, while our previously reported manganese complexes with the same ligands were active [6]. This observation brings up the hypothesis of a specificity of action of these molecules on yeasts and *Aspergillus* spp. on the one hand and dermatophytes on the other.

Additionally, the cobalt complexes showed a good activity on the two *Scedosporium* species i.e. *S. apiospermum* which ranks the second among the filamentous fungi colonising the airways of patients with cystic fibrosis [29] and *S. prolificans* causing severe disseminated infections in leukemic patients [30].

Here, the ligands seem to be involved in these results as suggested by their growth inhibition found versus the same strains. However, their activity was always less pronounced than for the complexes, indicating that complexation with cobalt enhances the antifungal activity of the ligands. This can be explained by chelation theory: chelation reduces the polarity of metal ion mainly because of partial sharing of its positive charge with the donor groups. Also, chelation increases the lipophilic nature of the metal ion which subsequently favours its permeation through the lipid layer of the cell membrane [31, 32]. These last results are quite

Table 3 Antifungal activities of ligands and complexes against pathogenic fungal strains, results correspond to the diameter of the inhibition zone (mm)

	H ₂ L ¹	H ₂ L ²	H ₂ L ³	[CoL ¹ (H ₂ O) ₂]	[CoL ² (H ₂ O) ₂]	[CoL ³ (H ₂ O) ₂]	Amphotericine B	Itraconazole
<i>A. flavus</i>	0	0	0	14	0	16	14	18
<i>A. fumigatus</i>	0	0	0	18	14	16	30	0
<i>A. terreus</i>	0	0	0	13	0	20	11	0
<i>C. albicans</i>	0	0	0	23	14	22	30	22
<i>C. glabrata</i>	0	14	0	25	15	27	25	20
<i>C. parapsilopsis</i>	0	0	0	36	28	33	25	35
<i>C. tropicalis</i>	0	16	13	23	18	17	15	27
<i>M. canis</i>	0	0	14	0	0	18	25	40
<i>M. gypseum</i>	0	18	15	0	0	0	25	25
<i>M. persicolor</i>	20	18	15	0	0	16	20	30
<i>S. apiospermum</i>	23	22	18	30	16	35	14	20
<i>S. prolificans</i>	22	20	14	38	23	27	0	0
<i>T. mentagrophytes</i>	0	15	16	0	0	35	24	30
<i>T. tonsurans</i>	0	20	16	0	0	13	25	25

interesting because these fungi are usually poorly susceptible to current antifungals including Amphotericin B and Itraconazole chosen as a reference in this study.

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