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Oxovanadium(IV) complexes of hydrazides: Potential antifungal agents

ZAHIDA T. MAQSOOD[1], KHALID M. KHAN[2], UZMA ASHIQ[1], RIFAT A. JAMAL[1], ZAHID H. CHOHAN[3], MOHAMMAD MAHROOF-TAHIR[4], & CLAUDIU T. SUPURAN[5]

[1]*Department of Chemistry, University of Karachi, Karachi 75270, Pakistan,* [2]*H.E.J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi 75270, Pakistan,* [3]*Department of Chemistry, Bahauddin Zakariya University, Multan, Pakistan,* [4]*Department of Chemistry, St Cloud State University, 720 Fourth Avenue S. 358 Math and Science Center, St Cloud, MN 56301, USA, and* [5]*Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, University of Florence, Via della Lastruccia 3, Rm. 188, Polo Scientifico 50019 Sesto Fiorentino (Firenze), Italy*

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Abstract

Oxovanadium(IV) -derived antifungals have been prepared by condensing equimolar amounts of vanadyl sulfate with hydrazides. All the synthesized ligands and their metal complexes were characterized by IR, UV–Visible and micro analytical data. These synthesized compounds were screened for their antifungal activity against *Aspergillus flavus (A. flavus)*, *Trichophyton longifusus (T. longifusus)*, *Candida albicans (C. albicans)*, *Microsporum canis (M. canis)*, *Fusarium solani (F. solani)* and *Candida glaberata (C. glaberata)* fungal strains. All complexes showed promising antifungal activity against different fungal strains with the exception of *F. Solani* and *C. glaberata*. Minimum Inhibitory Concentration (MIC) of different complexes and ligands are in the range of 250 to 400 μg/mL. Complex **7a** and ligand **13** exhibit lowest MIC of 250 μg/mL whereas, complex **5a** and ligands **2**, **7** and **14** showed highest MIC of 400 μg/mL.

Keywords: *Hydrazides, oxovanadium(IV) complexes, antifungal*

Introduction

Several studies have demonstrated that some anti-tumour drugs exhibited enhanced activity when administered in the form of their metal complexes [1,2]. In view of wide spread resistant strains of microorganism there is an urgent need for the development of new antifungal agents. The role of metal ions is stressed in many important processes and inorganic pharmacology has started to be a significant field with more than twentyfive inorganic compounds being used in therapy as antibacterial, antiviral and anticancer drugs. Recently, metal complexes of biomolecules have been used to design novel antibacterial/antiviral therapies targeted against, for example, human immunodeficiency (HIV) and human papilloma virus (HPV) infections. The hydrazides and their analogues are known to have several different biological activities such as tuberculostatic activity [3–5], antibacterial activity [6,7], antifungal activity [8,9], monoamine oxidase inhibitory activity [10–12], and antileishmanial activity [13].

Earlier, vanadium complexes with Schiff bases were reported to exhibit a range of biological activities including tuberculostatic activity, antibacterial activity and antifungal activity [14]. Over the past decade, numerous reports have been published on the insulin mimetic properties of vanadate and vanadyl both in *in vitro* and *vivo* environments [15–18]. In view of the various important biological activities of hydrazides and vanadium complexes we envisaged that a combination of hydrazide and vanadium within the

Correspondence: K. M. Khan, H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi 75270, Pakistan. Fax: 92 21 481901819. E-mail: hassaan2@super.net.pk

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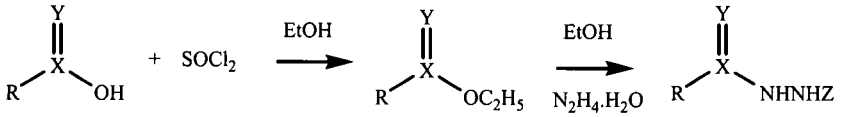

Compound	R	X	Y	Z
1	$-C_6H_5$	C	O	H
2	$2\text{-}OCH_3C_6H_4$	C	O	H
3	$4\text{-}OCH_3C_6H_4$	C	O	H
4	$2\text{-}NO_2C_6H_4$	C	O	H
5	$3\text{-}NO_2C_6H_4$	C	O	H
6	$4\text{-}NO_2C_6H_4$	C	O	H
7	$2\text{-}ClC_6H_4$	C	O	H
8	$3\text{-}ClC_6H_4$	C	O	H
9	$4\text{-}ClC_6H_4$	C	O	H
10	3-pyridyl	C	O	H
11	4-pyridyl	C	O	H
12	$-C_6H_5CH_2$	C	O	H
13	$-NH_2$	C	S	H
14	$2\text{-}CONHNH_2C_6H_4$	C	O	H
15	$4\text{-}CH_3C_6H_4$	SO	O	H
16	$4\text{-}OHC_6H_4$	C	O	H
17	$-OC_2H_5$	C	O	$-COOC_2H_5$
18	$-CH_3CHClCO$	C	O	H
19	$2\text{-}OHC_6H_4$	C	O	H
20	$3,4,5\text{-}OH(C_6H_2)$	C	O	H

Scheme 1. Synthesis of Hydrazides.

same molecule *i.e.* complex, the activities of hydrazides may be enhanced.

The present report describes a novel series of oxovanadium(IV) hydrazide complexes to explore their antifungal potential. Twenty hydrazides **1–20** alongwith twelve VO(IV) complexes (**1a–12a**) were synthesized. It was found that ligands **1–20** and complexes **1a–12a** showed varying degrees of

Table I. Physical, spectral and analytical data of ligands (**1–20**).

Compound	Ligand	M.P. (°C)	I.R. (cm^{-1})	C, H, N; Calc. (Found)%	Yield (%)
1	$(C_7H_8N_2O)$	117	3199, 1661, 1348, 1119	61.7 (61.4), 5.8 (5.3), 20.5 (20.2)	83
2	$(C_8H_{10}N_2O_2)$	80	3217, 1656, 1325, 1163	57.8 (57.6), 6.0 (6.1), 16.8 (16.5)	78
3	$(C_8H_{10}N_2O_2)$	136	3185, 1640, 1349, 1112	57.8 (57.5), 6.0 (6.4), 16.8 (16.9)	64
4	$(C_7H_7N_3O_3)$	125	3182, 1642, 1360, 1136	46.4 (46.2), 3.8 (3.9), 23.2 (22.9)	83
5	$(C_7H_7N_3O_3)$	147	3205, 1670, 1342, 1137	46.4 (46.2), 3.8 (3.9), 23.2 (23.5)	76
6	$(C_7H_7N_3O_3)$	218	3273, 1650, 1344, 1103	46.4 (46.7), 3.8 (3.4), 23.2 (22.9)	82
7	$(C_7H_7N_2OCl)$	118	3186, 1645, 1336, 1126	49.2 (48.4), 4.1 (4.3), 16.4 (16.7)	78
8	$(C_7H_7N_2OCl$	157	3196, 1664, 1339, 1116	49.2 (48.9), 4.1 (4.4), 16.4 (16.6)	75
9	$(C_7H_7N_2OCl$	163	3194, 1661, 1345, 1094	49.2 (49.5), 4.1 (4.2), 16.4 (16.1)	79
10	$(C_6H_7N_3O)$	163	3205, 1668, 1336, 1116	52.5 (52.3), 5.1 (5.3), 30.6 (30.3)	77
11	$(C_6H_7N_3O)$	173	3112, 1666, 1332, 1136	52.5 (52.7), 5.1 (5.4), 30.6 (30.5)	82
12	$(C_8H_{10}N_2O)$	116	3193, 1643, 1350, 1141	64.0 (64.2), 6.6 (6.3), 18.6 (18.4)	87
13	$(C_6H_5N_3S)$	183	3080, 1647, 1228, 993	13.1 (13.0), 5.4 (5.5), 46.1 (46.0)	75
14	$(C_8H_{10}N_4O_2)$	284	3013, 1660, 1373, 1080	49.4 (49.5), 5.1 (5.0), 28.8 (28.9)	80
15	$(C_7H_{10}N_2O_2S)$	110	3256, 1647, 1307, 1155	45.1 (45.1), 5.3 (5.2), 15.0 (15.0)	85
16	$(C_7H_8N_2O_2)$	266	3194, 1616, 1328, 1120	55.2 (55.2), 5.2 (5.1), 18.4 (18.3)	65
17	$(C_6H_{12}N_2O_4)$	134	3252, 1533, 1246, 1068	40.9 (40.8), 6.8 (6.8), 15.9 (15.8)	85
18	$(C_4H_7N_2O_2Cl)$	186	3131, 1613, 1418, 962	31.8 (31.7), 4.6 (4.7), 18.6 (18.6)	64
19	$(C_7H_8N_2O_2)$	135	3268, 1645, 1355, 962	55.2 (55.2), 5.2 (5.2), 18.4 (18.4)	54
20	$(C_7H_8N_2O_4)$	289	3298, 1650, 1340, 1103	45.6 (45.5), 4.3 (4.3), 15.2 (15.3)	87

Table II. Physical and analytical data of metal complexes.

Metal Chelates	M.P. (°C)	B.M. (μ_{eff})	C, H, N; Calc (Found) %	Yield (%)
1a	>300	0.931	31.3 (31.2), 3.3 (3.2), 10.4 (10.2)	56
2a	>300	0.692	32.2 (32.3), 3.6 (3.7), 9.3 (9.4)	67
3a	>300	0.897	32.2 (32.4), 3.6 (3.8), 9.3 (9.4)	63
4a	>300	0.832	26.8 (26.5), 2.5 (2.3), 13.4 (13.6)	62
5a	>300	0.969	26.8 (26.6), 2.5 (2.6), 13.4 (13.6)	62
6a	>300	0.949	26.8 (26.7), 2.5 (2.4), 13.4 (13.2)	71
7a	>300	0.866	27.7 (27.9), 2.6 (2.4), 9.2 (9.5)	58
8a	>300	0.939	27.7 (27.6), 2.6 (2.5), 9.2 (9.3)	64
9a	>300	0.948	27.7 (27.4), 2.6 (2.4), 9.2 (9.2)	64
10a	>300	0.963	26.7 (26.8), 2.9 (3.0), 15.6 (15.4)	59
11a	>300	0.965	26.7 (26.9), 2.9 (2.7), 15.6 (15.3)	63
12a	>300	0.895	34.0 (33.9), 3.9 (3.8), 9.9 (9.8)	67

antifungal activity against *A. flavus*, *T. longifusus*, *C. albicans*, *M. canis*, *F. solani* and *C. glaberata* fungal strains.

Material and methods

All the hydrazides were synthesized according to the literature [13] and were crystallized by methanol. Vanadyl sulphate penta hydrate ($VOSO_4 \cdot 5H_2O$), solvents and all reagents were of analytical grade and used without purification. IR spectroscopic analyses were carried out on a Shimadzu-IR-460 spectrometer in KBr and values are reported in cm^{-1}. Elemental analyses was carried out on a Perkin Elmer 2400 CHN elemental analyzer. Electronic spectra were recorded on a Shimadzu-UV-1601 spectrophotometer in MeOH and DMSO solution. Magnetic measurements were made on powders by employing a Sherwood-manway-MSB Mk1 magnetic susceptibility balance using sealed off $MnCl_2$ solution as calibrant. Metal contents were determined by a iodometric titration and also confirmed using a 3100 Perkin Elmer Atomic absorption spectrophotometer. Sulphate contents were determined by a gravimetric method and analyzed as $BaSO_4$. Melting points were recorded on a SMP 10 Bibby Stuart Scientific apparatus and are uncorrected.

General method for the preparation of ligands (1–20)

Ethyl-4-nitrobenzoate (5.0 g, 25 mmol) was dissolved in ethanol (75 mL), and then hydrazine hydrate (5 mL, 100 mmol) was added and the mixture refluxed for 5 h. The solid obtained was washed with hexane to afford the hydrazide. Other ligands were prepared from their respective esters.

General method for the preparation of metal(IV) complexes (1a–12a)

A solution of $VOSO_4 \cdot 5H_2O$ (1.265 g, 5 mmol) in methanol (10 mL) was added slowly with stirring to a hot methanolic solution (15 mL) of 4-nitrobenzohydrazide (0.905 g, 5 mmol) and the reaction mixture refluxed on a water bath for 2 h, during which time the solid complex separated out. The reaction mixture was cooled to room temperature and the solid complex was filtered and washed with methanol to remove the unreacted metal salt and ligand. The product was dried over anhydrous $CaCl_2$.

Antifungal assay

All the synthesized ligands (**1–20**) and their respective metal VO(IV) chelates (**1a–12a**) were screened for *in vitro* antifungal activity against *A. flavus*, *T. longifusus*, *C. albicans*, *M. canis*, *F. solani* and *C. glaberata* using the agar tube dilution assay [19–21]. Test samples were dissolved in sterile DMSO to serve as stock solution. Sabouraud dextrose agar was prepared by mixing Sabouraud 4% glucose agar and agar agar in distilled water. It was then stirred with a magnetic stirrer for dissolution and a known amount was dispensed into screw capped test tubes. Test tubes containing media were autoclaved at 121°C for 15 min. Tubes were allowed to cool to 50°C and the test samples of the desired concentrations were taken from the stock solution into the non-solidified Sabouraud agar media. Tubes were then allowed to

Table III. Spectral data of the metal chelates.

Metal Chelate	I.R. (cm^{-1})	λmax (cm^{-1})
1a	1650, 1352, 1200, 959	23419, 17636
2a	1637, 1307, 1251, 977	23529, 17543
3a	1650, 1352, 1200, 959	23255, 17301
4a	1620, 1348, 1253, 972	23041
5a	1621, 1350, 1184, 959	22935, 18832
6a	1662, 1350, 1175, 959	22421, 17761
7a	1658, 1357, 1182, 955	23474, 17605
8a	1621, 1340, 1265, 987	23041, 19011
9a	1629, 1343, 1270, 1006	23094, 17699
10a	1654, 1365, 1200, 973	23201, 17761
11a	1618, 1364, 1210, 980	23310, 18281
12a	1658, 1367, 1200, 953	24630, 16181

solidify in a slanting position at room temperature. Each tube was inoculated with a 4 mm diameter piece of inoculum removed from a seven day old fungi culture. All culture containing tubes were inoculated at an optimum temperature of 28–30°C for growth for 7–10 days. Humidity (40% to 50%) was controlled by placing an open pan of water in the incubator. Cultures were examined at least twice weekly during the incubation. After incubation for 7–10 days, the test tubes with no visible growth of the microorganism were taken to represent the minimum inhibitory concentration (MIC) of the test samples, which were expressed in μg/mL. Miconazole and amphotericin B were used as standard drugs.

Results and discussion

Chemistry

The ligands (**1–20**) were prepared by refluxing hydrazine hydrate with the corresponding esters in ethanol (Scheme 1). The structures of these synthesized ligands were determined by spectroscopy and micro analytical data (Table I). All metal complexes of these ligands were prepared in same equimolar ratio.

In the solid state all the complexes are fairly stable in air so as to allow physical measurements. These complexes are very soluble in DMF and DMSO but insoluble in common organic solvents, are amorphous solids and have melting points >300°C. Elemental analysis and physical data (Table II)* for the complexes indicate that the reaction of the ligand with $VOSO_4 \cdot 5H_2O$ yielded binuclear complexes in which two metals are coordinated with two ligands while bridging through oxygen atoms of hydrazides. Spectral data for the metal chelates are presented in Table III.

Biology

All the synthesized ligands **1–20** and complexes **1a–12a** were screened for their antifungal activities and the results are shown in Table IV. The compounds were tested against six fungal strains, namely *Aspergillus flavus (A. flavus)*, *Trichophyton longifusus (T. longifusus)*, *Candida albicans (C. albicans)*, *Microsporum canis (M. canis)*, *Fusarium solani (F. solani)* and *Candida glaberata (C. glaberata)*. The results were compared with the standard drugs miconazole and amphotericin. Compounds which showed 80% or above growth inhibition were selected for minimum inhibitory concentration (MIC) studies. Compounds **1a, 2, 2a, 3, 4a, 5, 5a, 6, 6a, 7, 7a, 8a, 10, 11, 11a, 12a, 13, 14, 15** and **16** were selected for MIC against the respective strains. Compounds showed varying degrees of activity against almost all the tested fungi.

The present investigation indicates that most of the hydrazides **3, 5, 7, 8, 13, 15, 16, 17** along with their

Table IV. Results of antifungal bioassay (% inhibition).

Name of Fungi	Compound																							
	1	**1a**	**2**	**2a**	**3**	**3a**	**4**	**4a**	**5**	**5a**	**6**	**6a**	**7**	**7a**	**8**	**8a**	**9**	**9a**	**10**	**10a**	**11**	**11a**	**12**	**12a**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**SD***
Aspergillus flavus	00	**90**	**90**	00	90	00	00	**90**	90	50	**90**	00	**90**	00	00	00	00	00	00	00	00	**90**	30	**90**	00	**90**	00	00	00	00	00	00	**A**
Trichophyton longifusus	00	00	00	00	00	75	70	00	**100**	70	00	00	**90**	00	00	70	60	00	00	00	00	00	00	00	**90**	**90**	00	00	00	00	00	00	**B**
Candida albicans	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	**C**
Microsporum canis	00	00	00	**80**	**90**	60	40	00	50	**80**	00	**80**	70	**90**	70	**80**	50	00	**90**	00	**90**	00	50	00	**80**	00	**80**	**90**	50	00	00	00	**D**
Fusarium solani	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	50	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	**E**
Candida glaberata	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	**F**

*Standard drugs MIC μg/mL: **A** = Miconazole (70 μg/mL), **B** = Miconazole (110.8 μg/mL), **C** = Amphotericin B (20 μg/mL), **D** = Miconazole (98.4 μg/mL), **E** = Miconazole (73.25 μg/mL), **F** = Miconazole (110.8 μg/mL).

Table V. Minimum inhibitory concentration (μg/mL).

	Compound concentration (μg/mL)																
	1a	**2**	**2a**	**3**	**4a**	**5**	**5a**	**6**	**6a**	**7**	**7a**	**8a**	**10**	**11**	**11a**	**12a**	**13**	**14**	**15**	**16**	**SD***
Name of Fungi																					
Aspergillus flavus	360	400		380	350			350		400					300	325		400			**A**
Trichophyton longifusus						310				350							250	400			**B**
Microsporum canis			360	340			400		380		250	380	350	315			340		350	380	**C**

* Standard drugs MIC μg/mL: **A** = Miconazole (70 μg/mL), **B** = Miconazole (110.8 μg/mL), **C** = Miconazole (98.4 μg/mL).

oxovanadium(IV) complexes **3a, 5a, 7a, 8a** showed more significant antifungal activity against *M. canis*. The screening test showed that compound **4, 9, 10, 11** and **12** are potent against *M. canis* while their oxovanadium(IV) complexes **4a, 9a, 10a, 11a** and **12a** are inactive against this fungal strain.

It is revealed from the data that some hydrazides **2, 3, 5, 6, 7** showed strong growth inhibitory activity against *A. flavus* but their complexes **2a, 3a, 5a, 6a** and **7a** were found to be very weakly active or inactive against this fungal strain. In contrast some complexes **1a, 4a, 11a, 12a** showed significant activity but their ligands **1, 4, 11, 12** are inactive against *A. flavus*. From the present studies it is observed that compound **3a, 4, 5, 5a, 7, 8a** and **9, 13** and **14** displayed good antifungal profile against *T. longifusus*.

Compounds **1, 9a, 10a, 18–20** were found completely inactive against all the tested fungal strains.

Notably, some fungi, *C. albicans*, *F. solani* and *C. glaberata* were almost resistant to all tested compounds except compound **9** which exhibit 50% inhibition against *F. solani*.

The minimum inhibitory concentration (MIC) of eleven ligands and nine vanadium(IV) complexes were determined using the agar tube dilution assay [19–21]. The results are tabulated in Table V. The compounds were tested against three fungal strains, namely *A. flavus*, *T. longifusus* and *M. canis*. The MIC range of these selected vanadium compounds varied from 250–400 μg/mL (188–671 μM). In contrast, most of the ligands showed low inhibitory actvity. The compounds **1a, 2, 3, 4a, 6, 7, 11a, 12a,** and **14** have significant potential against *A. flavus*, whereas compounds **5, 7, 13** and **14** are active against *T. longifusus*. Some compounds **2a, 3, 5a, 6a, 7a, 8a, 10, 11, 13, 15** and **16** displayed a better antifungal profile against *M. canis*.

Note

*Maqsood, Z. T. et al., manuscript in preparation with detailed chemistry.

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