Effect of Platinum(II) Complexes of 4-Methoxy- and 4-Chlorobenzoic Acid Hydrazides on *Saccharomyces cerevisiae*

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Pt(II) Complexes, Biologically Active Ligands, Hydrazides, Yeast Susceptibility

This study reports the anti-yeast effect of the 4-methoxybenzoic acid hydrazide (pmbah), 4-chlorobenzoic acid hydrazide (pcbah) and their Pt(II) complexes: *cis*- $[PtL_2X_2]$ and *cis*-$[PtL(NH_3)Cl_2]$ where L is either pcbah or pmbah and X is Cl, Br or I. MICs of the 4-substituted analogues (20 000–625 μM) are much lower than those of the previously reported benzoic acid hydrazide and 3-methoxybenzoic acid hydrazide. Complex formation results in significant increase of potency which may be due to a change in the mechanism of action, but the MIC (>400–50 μM) and the IC_{50} (>400–1 μM) values show that higher activity of the ligands in the free state does not result in enhanced complex activity. Differences in the potency of iodo-, chloro- and bromo complexes suggest MIC and IC_{50} values may be in correlation with the stability of the complex, rather than with the activity of the free ligands. Osmotically unstable mutants were more susceptible to the compounds than their parent strains, but differences among the parent strains were greater.

Along with the continued design of platinum and other metal based carcinostatics (Lippert, 1992; Hydes and Russel, 1988) revived interest into the antimicrobial effect of transition metal complexes is observed. Bunker and James (1989) reported the possibility of using Pt-group metal complexes as antibacterial agents and that they were unable to isolate resistant strains towards the active compounds. Transition metal complexes of different biologically active ligands have been found to possess antifungal effect (Narang *et al.* 1990; Narang and Singh, 1985; Kutsenko, 1980). Recently, it was reported (Tabakova and Dodoff, 1995) that *cis*-Pt(II) complexes of benzoic acid hydrazide (bah) and 3-methoxybenzoic acid hydrazide (mmbah) exhibit higher anti-yeast effect than cisplatin (*cis*-diamminedichloroplatinum(II)). Scarcity of data on the structure-effect relationship for coordination compounds of antimicrobially active agents as well as the findings that bacteria can utilize some ligands as carbon and energy source upon complex decomposition (Bunker and James, 1989) stimulated us to investigate whether a ligand with higher potency in the free state would afford Pt complexes with enhanced activity. This study compares the growth-inhibiting effect of transition metal complexes of benzoic acid hydrazide derivatives with various potency in the free state. To this end we have selected series of Pt(II) complexes of 4-methoxybenzoic acid hydrazide (pmbah) and 4-chlorobenzoic acid hydrazide (pcbah). They differ only by the substituent or the site of derivatization and yet are 4 to 20-fold more active than the previously investigated bah and mmbah in the free state (Tabakova and Dodoff, 1995).

The anti-yeast effect was studied through the minimal inhibitory concentrations (MIC) and the concentrations, causing 50 per cent inhibition of yeast growth (IC_{50}). Individual values are presented in Table I. Identical strains and conditions as in the previous assay were used to allow comparison and arithmetic mean of the MICs and the IC_{50}s of the five strains for each of the complexes are presented in Table II.

Pmbah and pcbah inhibit yeast growth in concentrations of 2500 to 20 000 μM and 625 to 2500 μM respectively. They are 4-fold lower than those of bah and mmbah, except for some of the osmotically unstable mutants (cf. VY1160).

Platinum complexes had MICs ranging from 50 to >400 uM and IC_{50}s were from 1 to >400 μM. Invariably, complex formation results in significant decrease of both MIC and IC_{50} for the individual strains, but is not directly correlated with the activ-

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Table I. Inhibitory effect of 4-substituted benzoic acid hydrazides and their Pt(II) complexes on *Saccharomyces cerevisiae*, expressed by the MIC and IC_{50} values in μM.

	Strain									
	A364		E1278		VY481[c]		S288C		VY1160[c]	
Compound	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
pmbah[a]	2500	–	2500	–	5000	–	2500	–	20000	–
$[Pt(pmbah)_2Cl_2]$	100	68	200	60	100	55	100	60	100	45
$[Pt(pmbah)(NH_3)Cl_2]$	>400	350	>400	>400	>400	>400	400	270	400	170
$[Pt(pmbah)_2Br_2]$	400	285	>400	>400	>400	>400	400	275	400	200
$[Pt(pmbah)_2I_2]$	400	290	>400	>400	>400	>400	400	280	200	44
pcbah[b]	600	–	1250	–	2500	–	620	–	2500	–
$[Pt(pcbah)_2Cl_2]$	100	68	100	60	100	55	100	60	50	37
$[Pt(pcbah)(NH_3)Cl_2]$	200	147	200	110	400	275	200	137	50	15
$[Pt(pcbah)_2Br_2]$	100	69	100	55	100	55	100	80	100	1
$[Pt(pcbah)_2I_2]$	200	140	400	325	400	300	400	285	100	10
Cisplatin	800	120	800	100	800	70	400	200	200	10

[a] 4-Methoxybenzoic acid hydrazide.
[b] 4-Chlorobenzoic acid hydrazide.
[c] Sorbitol requiring osmotically unstable mutants.

Table II. Mean arithmetic values and ranges of MIC and IC_{50} of 5 strains of *Saccharomyces cerevisieae* for Pt(II) complexes with hydrazide ligands.

Complex		L[a]=bah[b]		L=mmbah[c]		L=pmbah[d]		L=pcbah[e]	
		mean	range	mean	range	mean	range	mean	range
$[PtL_2Cl_2]$	MIC	124	(200–20)	114	(200–20)	134	(200–100)	90	(100–50)
	IC_{50}	47	(70–13)	28	(80–6)	56	(68–45)	57	(68–37)
$[PtL(NH_3)Cl_2]$	MIC	94	(200–20)	114	(200–20)	>400	(>400–400)	210	(400–50)
	IC_{50}	41	(90–4)	47	(80–5)	>320	(>400–170)	137	(275–15)
$[PtL_2Br_2]$	MIC	130	(200–50)	114	(200–50)	>400	(>400–400)	100	(100–100)
	IC_{50}	46	(70–30)	27	(50–5)	>313	(>400–200)	52	(80–1)
$[PtL_2I_2]$	MIC	440	(800–200)	440	(800–200)	>400	(>400–200)	300	(400–100)
	IC_{50}	74	(120–30)	116	(200–20)	>275	(>400–44)	210	(350–1)

[a] L = hydrazide ligand.
[b] Benzoic acid hydrazide.
[c] 3-Methoxybenzoic acid hydrazide.
[d] 4-Methoxybenzoic acid hydrazide.
[e] 4-Chlorobenzoic acid hydrazide.

ity of the free ligands. Least active are the pmbah series, although in the free state the compound is more potent than bah and mmbah. Pcbah containing complexes have lowest MICs, while mmbah have lowest IC_{50}s. Chlorocomplexes were found to be more active than bromo- and iodo-ones, regardless of the organic ligand. Considering the arithmetic mean values for MIC and IC_{50}, it appears that complex activity depends rather on the nature of the anionic ligand, than on the potency of the organic one and may be associated with the stability of the complex. Probably, the amount of ligand released upon complex dissociation is too small to affect MIC and IC_{50}. Along with other factors (Galgiani and Stevens, 1976) complex inactivation rate may account for lack of coincidence between MICs (read at the 48th h) and IC_{50}s (for the 12th or 16th h).

All the investigated complexes fall within the order of activity of Cisplatin and is likely that they act by DNA platination. The findings of Brown *et al.* (1993) may explain the fact that the suscep-

tibility of osmotically unstable mutants was closer to that of their parent strains, than to that of other sorbitol-requiring yeasts.

In conclusion, the more potent benzoic acid hydrazide analogues did not afford *cis*-Pt(II) complexes of higher anti-yeast effect as estimated by the MIC and IC_{50} values. *Cis*-Pt(II) complexes of pcbah and pmbah had much higher growth inhibiting effect than the respective free hydrazides. Complex activity was observed to depend on the anionic ligand and is probably associated with complex stability.

Experimental

The ligands pcbah and pmbah have been synthesized according to Struve (1894) and Sah and Chang (1936). The *cis*-platinum complexes of pcbah and pmbah were prepared as described by Dodoff *et al.* (1995). Cisplatin was prepared according to Spassovska *et al.* (1981). Yeast susceptibility was studied by the MIC and the IC_{50} values. Briefly, fresh DMSO solutions 40–4000 μM of the complexes or 800–200 000 μM of the free ligands compounds were added to Sabouraud nutrient medium complemented with 10% sorbitol, the DMSO:broth ratio being 1:10 v/v for each dilution (Final concentrations of the Pt complexes were 4–400 μM and of pmbah or pcbah – 80 to 20 000 μM.

MICs were determined by the two-fold broth dilution method (Reiner, 1982), the final inoculum size being $1x10^5$ culture forming units per ml. Visual readings were made after 48 h of incubation at 30 °C. In this concentration DMSO had no visually observable growth inhibiting effect. All assays were performed independently three times and values in Table I are the arithmetic mean of the figures from the separate tests for each strain. The mean minimal inhibitory concentration of a compounds for the five strains investigated have been obtained by summing the five individual MICs for each strain and dividing this sum into five (Table II). Susceptibility range is indicated instead of the standard deviation due to the small number of strains tested. IC_{50} were extrapolated from growth inhibition (in per cent) *vs.* concentration curves (Galgiani *et al.,* 1976) for the 12th or 16th (for the osmotically unstable mutants) hours after inoculation. I,% was calculated as $(A_c\text{-}A_i):A_c x100$, A_c and A_i being the optical densities of the cultures at 520 nm, grown in the presence of 0, 8, 80 and 400 μM of the compounds respectively, prediluted in DMSO, which ensured equal broth:DMSO ratio of 1:20 (v/v), including the control test cultures. Initial optical density of the cultures was 0.05 and after the set time this variable (A_c) ranged from 0.4 to 1.0 for the different cultures, containing only DMSO in the broth in 1:20 (v/v).

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