Metal Nitrosyls as Antimicrobial Agents

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Synopsis. Five known nitrosyl complexes of Fe and Co have been evaluated for their antiviral, antibacterial, and antifungal activities.

Although the use of metal complexes in the control of plant and animal diseases have been well documented in the literature,[1-4] yet no definite structure-activity correlationship has been established. Initial studies, however, indicate that the minor change in the structure of complex is sometime associated with a major change in its biological activity.[4] In view of this, together with our interest in the synthesis of metal nitrosyls, the few nitrosyls have been synthesized and screened for their antiviral and antibacterial activities which have been correlated with their structures.

Experimental

Synthetic and microanalytical work has been carried out in the chemistry department of the Indian Institute of Technology, Kanpur. All of the metal nitrosyls were synthesized by the known procedure[5-7] and are reported in Table 1 along with their physical data. Antimicrobial screening has been carried out in the pharmaceutical science department of University of Antwerpen, Belgium.

EPTT technique[8] was used to screen the complexes for their antiviral activity. Monolayers of virocells on microtiter plates were injected with 0.1 ml of a ten-fold diluted solution of the virus suspension, obtained after 60 hs incubation at 37 °C. The cytotoxicity and virus growth control were determined on the same microtiter plates after incubation for several days at 37 °C in a humid incubator. The cytopathic effect was determined by light microscopy while the antiviral activity as viral titer reduction factor (RF).

Antibacterial activity of the compounds was determined by agar diffusion technique. Agar plates were homogeneously inoculated, 5 holes (7 mm ϕ) were prepared in the agar and 0.2 ml of the solution of each compound was placed in the holes [reference antibacterium, neomycin 500 $\mu g\,ml^{-1}$, nystatine 5000 $\mu g\,ml^{-1}$ for Candida sps]. The plates were first incubated at 4 °C for one hour and then for 18 hs at 30 °C. The diameter of the inhibition zone (IZ) was measured around each hole. The ratios of (IZ) of compounds and (IZ) of reference were then calculated.

The same agar diffusion technique was used for measuring the antifungal activity of the compounds. In the culture medium fungal cultures maintained in a slightly acidic nutrient broth (NB) [25 ml. 1M HCl+750 M NB] (1 M=1 $mol\,dm^{-1}$), were shaken for a long time and the mycelium was homogenized by ultrasonication. Saturated dextrose agar (gipco Europe) was distributed in the growth control tubes (2—2.5 ml/test tube) and was autoclaved. Agar was liquified at 45 °C before use. After liquification 0.25 mg of the compound was thoroughly mixed with the agar in the growth control tube. The mycelium was homogeneously spread all over the agar surface and was incubated at the ambient temperature for two weeks. Similar agar growth tubes were prepared for comparison without the addition of compounds in them. The fungal growth in the control tube was then distinguished from that of the tube containing culture medium and inhibition was then calculated as described above for antibacterial activity.

Results and Discussion

The results given in Table 2 suggest that the compound A_1 is of significant antiviral interest. Cobalt complex (A_1) has a square pyramidal geometry around Go(II) ion with nitrosyl as a ligand in the apical position.[9] It, being coordinatively unsaturated, may have a tendency for further coordination leading to the octahedral environment around the metal center. Furthermore, it has been reported that the metal chelates show their antiviral activity (preventing or slowing down the viral multiplication) eithar by (1) the direct absorption of the metal chelates and, thus, reducing their multiplication or (2) by the primary action of the metal chelate on the host cells.[10]

Since it has already been reported[10] that *Herpes*

Table 1. Analytical and IR Spectral Data of the Compounds Tested

Compound	IR ν_{NO} /cm^{-1}	C	H	N	S
(A_1) [Co(NO)salen]	1640	45.1 (45.7)	3.8 (4.2)	15.8 (15.2)	—
(A_2) [Fe(cyst)$_2$NO]	1625	24.3 (25.77)	3.8 (4.3)	11.00 (10.02)	23.0 (22.9)
(A_3) [Fe(NO)(phen)$_2$][Fe(NO)(mnt)$_2$]	1820	47.2 (47.4)	2.0 (1.7)	15.5 (15.7)	15.5 (14.9)
(A_4) [Fe(NO)(bpy)$_2$][Fe(NO)(mnt)$_2$]	1620	37.4 (37.2)	2.1 (2.3)	16.6 (16.9)	—
(A_5) [Fe(NO)mnt(tpp)$_2$]	1620	68.7 (69.2)	3.9 (4.2)	6.3 (6.08)	9.7 (10.1)

salen=*N*,*N*′-Disalicylidene ethylenediamine, mnt=1,2-Dicyano-1,2-ethylenedithiolato, cyst=Cysteine, bpy=2,2′-Bipyridyl, phen=1,10-Phenanthroline, tpp=Triphenylphosphine.

Table 2. Antiviral Activity

Compounds tested	*Poliomyelitis*		*Coxackie*		*Semliki forest*		*Herpes simplex*	
	Concn /ml	(RF)	Concn /ml	(RF)	Concn /ml	(RF)	Concn /ml	(RF)
(A_1) [CoNO(salen)]	50	10^3	50	10^2	50	10	50	10
	25	20	25	10	25	10	—	—
(A_2) [Fe(cyst)$_2$NO]	50	1	50	1	50	1	50	1
(A_3) [Fe(NO)(phen)$_2$][Fe(NO)(mnt)$_2$]	25	1	25	1	25	1	25	1
(A_4) [Fe(NO)(bpy)$_2$][Fe(NO)(mnt)$_2$]	50	1	50	1	50	1	50	1
(A_5) [Fe(NO)(mnt)(tpp)$_2$]	50	1	50	1	50	1	50	1

Antibacterial Activity

Compound	Microorganism		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*
(A_1) [Co(NO)salen]	0	0.77	0.80

simplex virus (HSV) gets inactivated by the direct absorption of the metal chelates, therefore the poor activity of the complexes against HSV could be understood in terms of the direct absorption mechanism. But the significant activity of Co(II) complex (A_1) against *Poliomyelitis* virus indicates that same mechanism will not be operative in this case. Thus the only alternative mechanism may be the primary action of the metal complex on the host cell. The primary action on the host cells by the five coordinated metal complexes may occur in the following two ways: (1) Coordinatively unsaturated Co(II) complexes pick up an oxygen molecule[9] from the system in its sixth position and thus competes with the host cells. This will result in the depletion of oxygen for the host cells leading to starvation and thus preventing the viral multiplication. (2) The viral components (nucleic acids) themselves may act as a ligand for the sixth coordination site of the Co(II) complex resulting in preventing the viral multiplication. In case the latter alternative was operative in our complexes, all the complexes used in the testing should have shown at least some antiviral activity. Thus we assume that the absence of any activity in other cases indicate the first alternative to be operative.

The complexes of iron listed in Table 1 are definitely not following the mechanism of coordinative unsaturation because of their being inactive, in spite of the availability of the vacant sites for further bond formation. One can reason out for their inactivity due to very little penetrating power in the host cells, thus showing their inability to deprive the virus of the metabolites and energy sources required for its multiplication.[11]

Out of all five complexes tested for antibacterial activity, only the Co(II) complex (A_1) (Table 2) showed significant activity against *Pseudomonas aeruginosa* and *Escherichia coli* where the mode of action of the complex could also be explained on the same ground as is explained for its antiviral activity. All iron complexes were found to be inactive against all the test bacteria.

These five complexes were also tested for their antifungal activity against *Aspergillus flavus, A. fumigatus, A. niger,* and *Tricophyton mentagrophytes* but none of them were found active.

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