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PHOTOACTIVATED VIRUCIDAL PROPERTIES OF TRIDENTATE 2,2'-DIHYDROXYAZOBENZENE AND 2-SALICYLIDENEAMINOPHENOL PLATINUM PYRIDINE COMPLEXES

Gregory D. Cuny,[1a]* Kevin D. Landgrebe,* and Terrance P. Smith[1b]

3M, 3M Center, St. Paul, MN 55144-1000, U.S.A.

Michael J. Fehr and Jacob W. Petrich

Department of Chemistry, Iowa State University, Ames, IA 50011, U.S.A.

Susan Carpenter

Department of Microbiology, Immunology, and Preventive Medicine, Iowa State University, Ames, IA 50011, U.S.A.

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Abstract: The potent photoactivated virucidal activity of tridentate 2,2'-dihydroxyazobenzene- and 2-salicylideneaminophenol platinum pyridine complexes **1**, **2**, **4**, **6**, **7**, **9**, and **10** against enveloped viruses (e.g., EIAV, HIV, and HSV) is described.

The effects from light interacting with organic and inorganic materials on living organisms have been the subject of research for nearly a century.[2] The application of light-absorbing compounds in medicine has provided an additional mode of therapy available to clinicians for the treatment of many cancers.[3] For example, hematoporphyrin derivative has been successfully utilized in photodynamic therapy for treating an array of malignancies.[4]

Recent reports[5a] have described efforts to extend photodynamic therapy to combat viral contamination and infections with an assortment of structurally diverse photoactivated materials, including hypericin,[5b] rose bengal,[5c] merocyanines,[5d] 1,8-naphthalimides,[5e] and psoralens.[5f] The utilization of photosensitizers has been reduced to practice for the sterilization of whole blood and blood components.[6] We now report that some tridentate 2,2'-dihydroxyazobenzene and 2-salicylideneaminophenol platinum pyridine complexes under ordinary laboratory light conditions exhibit potent virucidal activity against enveloped viruses such as equine infectious anemia virus (EIAV), human immunodeficiency virus type 1 (HIV-1), and herpes simplex virus type 1 (HSV-1).

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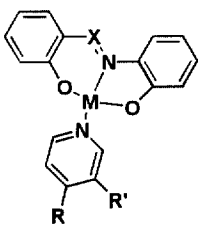

Compound	M	X	R	R'	log FFU/mL of EIAV $10^{a,b}$	$1.0^{a,b}$	$0.1^{a,b}$	$10^{a,c}$
1	Pt	N	vinyl	H	0	0	3.2	3.7
2	Pt	N	CH_2OH	H	0	0	3.4	3.8
3	Pt	N	$CH(C_4H_9)_2$	H	4.2	ND[d]	4.7	ND[d]
4	Pt	N	Et	H	0	2.5	3.6	3.9
5	Pt	N	CHO	H	2.5	3.7	3.7	3.9
6	Pt	N	H	H	0	0	3.9	3.9
7	Pt	N	H	CH_2OH	0	0	4.4	4.7
8	Pt	N	H	CO_2Et	3.1	ND[d]	ND[d]	4.3
9	Pt	CH	vinyl	H	0	0	ND[d]	4.4
10	Pt	CH	CH_2OH	H	0	0	4.4	4.9
11	Pd	N	vinyl	H	4.1	ND[d]	4.1	ND[d]
12	Pd	N	Et	H	4.1	ND[d]	4.3	ND[d]
13	Ni	N	vinyl	H	4.4	ND[d]	4.3	ND[d]

(a) μg/mL; (b) in the light; (c) in the dark; (d) not determined.

Table 1

The metal pyridine complexes **1-13** were prepared by allowing 2,2'-dihydroxyazobenzene or 2-salicylideneaminophenol to react with a metal halide salt (e.g., K_2PtCl_4, K_2PdCl_4, or $NiCl_2$) in the presence of base (K_2CO_3 or NaOEt) and an excess of pyridine ligand.[7]

The photoactivated virucidal properties of the metal complexes were evaluated against the enveloped RNA-containing lentivirus EIAV that serves as a model for the structurally, genetically, and antigenically related HIV.[8] Solutions of the metal complexes and the virus (ca. 10^4 viral units/mL) were exposed to ordinary laboratory light (irradiance = 270 $\mu W/cm^2$, emission from 402-677 nm with intense spikes at 435, 535, 560, and 571 nm) for 30 min and control samples were prepared in the dark. Samples were serially diluted, inoculated onto equine dermal cells and virus infectivity (log FFU/mL; FFU = focal forming units) was determined using a focal immunoassay as previously described.[9]

All of the metal complexes tested as dark controls were inactive. Platinum complexes **1**, **2**, **6**, **7**, **9**, and **10** completely inactivated the virus at 1 μg/mL, while **4** required 10 μg/mL (Table 1).[10] Platinum complexes that contained electron withdrawing substituents on the pyridine ligand (e.g., **5** and **8**) were inactive at the concentrations tested. In addition, complex **3** containing a large hydrophobic substituent on the pyridine ring was also inactive at the concentrations evaluated. The palladium complexes **11** and **12** and the nickel derivative **13** were inactive at the concentrations tested. Furthermore, platinum complexes **1**, **6**, and **10** were found to

exhibit potent photoactivated virucidal activity against HIV-1 (enveloped RNA-containing virus) and HSV-1 (enveloped DNA-containing virus) at 10 μg/mL, while **2** was active at 1.0 μg/mL.[11] The testing protocol was similar to the method utilized for EIAV (vida supra), except that the reduction in virus titer was determined.[12]

Preliminary experiments indicated that **1** was inactive against the nonenveloped encephalomyocarditis virus under similar lighting conditions.[13] This finding suggests that the mechanism of action for the metal complexes described herein may intimately involve the lipid bilayer membrane or its associated glycoprotein components. At the present time, however, other mechanisms of action can not be discounted such as photoinduced modification of the nucleic acid portion of the virus.

Singlet oxygen has been implicated in the mechanism of virus inactivation for many photosensitizers.[5] Upon exposure to light and aerobic conditions the virucidal complexes **1**, **4**, **6**, and **10** generated singlet oxygen, while the nonvirucidal complex **5** generated singlet oxygen to a lesser extent (Table 2).[14, 15] The nonvirucidal palladium complex **11** and nickel complex **13** did not generate singlet oxygen utilizing the same experimental conditions. Therefore, it seems likely that the production of singlet oxygen by some of the platinum complexes upon illumination is necessary for virus inactivation. However, the precise role of singlet oxygen, generated by the metal complexes described herein, on virus inactivation remains to be elucidated.

Compound	$^1O_2$ production[a]
1	0.39
4	0.33
5	0.10
6	0.36
10	0.99
11	<0.01
13	<0.01

(a) number of equivalents of 6-hydroxy-2H-pyran-3(6H)-one produced from exposure of 1 equivalent of furfuryl alcohol to ordinary laboratory light (irradiance = 270 mW/cm^2, emission from 402-677 nm with intense spikes at 435, 535, 560, and 571 nm) for 24h in the presence of compounds **1**, **4-6**, **10**, **11**, or **13**; see ref 15.

Table 2

In summary, we have shown that some tridentate 2,2'-dihydroxyazobenzene and 2-salicylideneaminophenol platinum pyridine complexes under subdued light conditions exhibit potent virucidal activity against several enveloped viruses. Furthermore, substituents on the pyridine ligand have been shown to substantially effect the virucidal properties of the platinum complexes described herein. Currently, the photoinduced biological activity of these materials on prokaryotic and eukaryotic cells is being evaluated.

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References and Notes

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11. Compounds **2**, **7**, and **9** have not been evaluated against HIV-1 or HSV-1 to date.
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15. Complex **1**, **4-6**, **10**, **11**, or **13** (0.33 equiv.) in CD_2Cl_2 were exposed to ordinary laboratory light (irradiance = 270 $\mu W/cm^2$, emission from 402-677 nm with intense spikes at 435, 535, 560, and 571 nm) for 24h in the presence of furfuryl alcohol (1.0 equiv.), a known singlet oxygen trap. The relative quantity of singlet oxygen generation was determined by measuring the production of 6-hydroxy-2*H*-pyran-3(6*H*)-one. See: Kuo, Y. -H.; Shih, K. -S. *Heterocycles* **1990**, *31*, 1941.